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THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The year 1948 from a financial point of view was quite satisfactory. Increased use of the Laboratory's facilities and continued progress in the program of repairs and additions to buildings and equipment were reflected in considerably increased expenditures. The total paid out for current expenses was $264,897.87. The amount spent for additions to capital assets, including $22,856.84 paid on the new boats, was $53,814.81, so that total expenditures were $318,712.68.

Fortunately, current receipts also increased to a total of $291,044.89. To this sum was added $26,539.78 in special, non-recurring receipts that made a total of $317,584.67, so that on a cash basis, the deficit for the year was only $1,128.01. These special receipts consisted of $4,708.11 from the sale of securities given in recent years by Mrs. W. Murray Crane for current expenses and applied with her permission towards the purchase of the "Dolphin," $6,218.88 balance remaining in the Reserve Fund, $9,300.00 given by the American Cancer Society and used chiefly for new equipment, and $6,312.79 balance remaining in the Boat Fund contributed in 1947 and 1948.

The year was also notable for the receipt of 500 shares of Crane Company common stock, valued at $17,250.00 from the Estate of Dr. Frank R. Lillie, and the grant from the Rockefeller Foundation of $250,000., $100,000. for current expenses over a period of five years, and $150,000. for the restoration of "Old Main."

As in recent years, the accounts have been audited by Seamans, Stetson & Tuttle, certified public accountants of Boston, and a copy of their report is available for inspection. The "Balance Sheet" as prepared by them is appended as Exhibit A. As of December 31, 1948, the total *book* value of all the Endowment Assets was $979,865.18, an increase for the year of $1,188.51. The *market* value of these assets was $988,639.62, a reduction for the year of $1,174.54. Plant Assets (Land, Buildings and Equipment) amounted to $1,133,168.64, an increase of $21,217.09.

Following last year's precedent, the "Statements" which follow, prepared with the assistance of Mr. Homer P. Smith, Assistant Business Manager, give the actual financial transactions of the year on a cash basis excluding accruals, non-cash items, depreciation, interdepartment charges, etc. They are:

I. Summary Cash Statement for Year,
II. Current Expenses by Departments,
III. Additions to Capital Assets from Current Funds,
IV. Retirement Fund,
V. Fellowship Fund,
VI. Special Funds,
VII. Real Estate Accounts,
VIII. Agency Accounts.

I. Summary Cash Statement for Year Ended December 31, 1948

		Expenditures		
	Receipts	Current	Additions to Capital Assets	Total Expenditures
Membership Dues	$ 2,268.00			
Donations for Current Expenses[1]	1,815.00			
Income from Endowment	40,707.09			
Income from Other Securities	23,768.60			
Real Estate Rentals	6,360.00	$ 986.20		$ 986.20
Instruction	12,403.64	8,357.17		8,357.17
Research (incl. Apparatus and Chemical Dep'ts)	25,673.04	26,307.31	$11,346.18	37,653.49
Mess	38,858.06	39,375.21	417.13	39,792.34
Dormitories and Apt. House	16,788.30	14,175.34	327.71	14,503.05
Library[2]	5,989.85	9,431.37	7,983.41	17,414.78
Buildings and Grounds		42,276.09	4,367.57	46,643.66
Supply Department[3]	106,724.12	85,693.13	29,372.81	115,065.94
"Biological Bulletin"	7,538.15	8,345.89		8,345.89
Administration		27,652.96		27,652.96
Miscellaneous	2,151.04	2,297.20		2,297.20
	$291,044.89	$264,897.87	$53,814.81	$318,712.68

Special Receipts		
Sale of Securities	$4,708.11	
Reserve Fund	6,218.88	
American Cancer Soc.	9,300.00	
Boat Fund	6,312.79	26,539.78
		$317,584.67

Total Expenditures	$318,712.68
Total Receipts	317,584.67
Cash Deficit for Year	$ 1,128.01

Notes:

[1] Donations were $1,320. given by the "Associates" of the Laboratory and $495. miscellaneous contributions.

[2] The Library income consists of $3,000. payment from the Oceanographic Institution towards Library expenses and $2,989.85 from the Carnegie Book Fund (balance of Book Fund still available is $11,416.15). The monetary value of serials received in exchange for the "Bulletin," estimated at $3,571.26, is not included, nor is the $800. received from the Oceanographic Institution for the purchase of books for their account.

[3] The actual 1948 sales of the Supply Department were $94,701.77, a slight decrease from the 1947 sales. The values of specimens and supplies furnished the Research and Instruction Departments were $8,817.50 and $5,488.82 respectively. If these values are taken into account, and also the gain in inventory of $6,042.36, the decrease in accounts receivable of $14,101.28, and a debit charge of $2,100. for administration and maintenance expense, there would be a net profit of $25,178.39 on the operations of the Supply Department for 1948. This does not take into account the $6,515.97 spent for capital items, exclusive of the new boats, or the auditors' charges of $1,684.38 for depreciation. If these had been included, the net profit for the Supply Department would have been $16,978.04.

II. Current Expenses for 1948 by Departments

Administration

Salaries	$ 21,661.02
Central Hanover Bank Trustee Commissions	1,036.22
Falmouth Bank Charges	154.53
Audit	1,162.35
Treasurer's Office	600.00
Advertising	374.52
Office Supplies	1,320.08
Sundries (Tel., Postage, etc.)	1,803.82
	28,112.54
Deduct Cash Receipts	459.58
	27,652.96

Instruction

Salaries and Travel	8,210.40
Sundries	146.77
	8,357.17

Research (incl. Apparatus and Chemical Dep'ts)

Salaries	14,316.12
Travel	200.00
Repairs	344.66
Supplies and Sundries	13,245.80
	28,106.58
Deduct Cash Receipts	1,799.27
	26,307.31

Library

Salaries	8,947.57
Office Supplies	190.08
Sundries	333.38
	9,471.03
Deduct Cash Receipts	39.66
	9,431.37

Buildings and Grounds

Salaries and Wages	25,253.60
Fuel	3,711.08
Gas	1,557.45
Light and Power	3,320.66
Water	722.29
Insurance	1,706.71
Repairs	4,329.09
Sundries	2,043.13
	42,644.01
Deduct Cash Receipts	367.92
	42,276.09

Dormitories and Apt. House

Salaries and Wages	5,576.27
Lighting, Gas and Water	2,114.74
Repairs	3,082.81
Outside Rentals	500.00
Laundry	1,455.41
Insurance	762.22
Sundries	716.17
	14,207.62
Deduct Cash Receipts	32.28
	$ 14,175.34

Mess

Salaries and Wages	$ 8,800.84
Cost of Food	25,989.86
Gas, Water, Light and Power	2,151.89
Repairs	89.48
Replacements of Dishes, etc.	647.78
Insurance	623.98
Laundry	327.23
Freight and Express	58.08
Sundries	780.16
	39,469.30
Deduct Cash Receipts	94.09
	39,375.21

Supply Department

Salaries and Wages	33,607.64
Purchase of Specimens	32,529.14
Chemicals	2,384.83
Containers	3,826.05
Boat Expenses	2,631.38
Truck Expenses	559.94
Freight and Express	3,107.31
Fuel, Light and Power	809.35
Office Supplies	460.08
Telephone and Telegraph	206.29
Insurance (incl. Boats)	2,381.84
Advertising	392.76
Specimens and Supplies purchased for Research Dep't	1,035.64
Nets, Floats, etc.	461.00
Repairs and Sundries	1,349.08
	85,742.33
Deduct Cash Receipts	49.20
	85,693.13

"Biological Bulletin"

Salaries	2,130.00
Printing, etc.	6,222.84
	8,352.84
Deduct Cash Receipts	6.95
	8,345.89

Real Estate (Leased Out)

Taxes and Insurance on Bar Neck Property (Garage) and Janitor's House	986.20

Other Expenses

Workmen's Compensation Insurance	1,004.67
Truck Expense	462.99
Bay Shore and Great Cedar Swamp Expenses	256.47
Interest on Mortgage	406.67
Evening Lectures	166.40
	2,297.20
Total Expenses	$264,897.87

III. Additions to Capital Assets from Current Funds

A. Buildings

Brick Laboratory	$ 2,247.59
Supply Dep't	6,318.12
Mess	202.27
	$ 8,767.98

B. Library

Back Sets	$ 1,903.37
Books	569.15
Serials	2,989.39
Reprints	13.75
Binding	2,507.75
	$ 7,983.41

C. Equipment

Apparatus Dep't	$ 9,069.71
Dormitories	327.71
Mess	214.86
Brick Laboratory	306.97
Carpenter Shop	421.42
Boats	22,856.84
Supply Dep't	197.85
Truck	1,391.59
Machine Shop	2,276.47
	$37,063.42
Total Additions to Capital Assets	$53,814.81

IV. Retirement Fund (Securities and Cash)

Jan. 1, 1948, Balance on Hand		$16,388.44
Receipts:		
Income and Principal Gains	$ 243.02	
Payment from M.B.L. (10% of 1948 Payroll)	7,323.62	7,566.64
		23,955.08
Disbursements:		
Pensions paid in 1948	5,710.00	
Custodian Fees	50.00	5,760.00
Dec. 31, 1948, Balance on Hand		$18,195.08

V. Fellowship Fund

Jan. 1, 1948, Balance on Hand		$ 1,043.03
Receipts:		
Payment from Lalor Foundation	$ 5,000.00	5,000.00
		6,043.03
Disbursements:		
Fellowships (nine)	3,665.35	
Laboratory Space, Apparatus and Supplies	1,499.53	5,164.88
Dec. 31, 1948, Balance on Hand		$ 878.15

VI. Special Funds

A. Boat Fund

Jan. 1, 1948, Balance on Hand		$ 5,302.79
Receipts:		
Contributions	$ 1,010.00	1,010.00
		6,312.79
Disbursements:		
Transfer to M.B.L. Income Account for New Boats		6,312.79
		– 0 –

B. Dr. Frank R. Lillie Memorial Fund

Jan. 1, 1948, Balance on Hand (Initial Contribution from Dr. G. H. A. Clowes)	$ 1,000.02
No Transactions in 1948	

VII. Real Estate Accounts

A. Devil's Lane Property

Cash Received in 1948 from Sales	$ 4,097.50
Disbursements (Taxes)	193.30
Book Value of Unsold Lots, Dec. 31, 1948	36,739.43
Accounts Receivable, Dec. 31, 1948	6,662.00

B. Gansett Property

Cash Received in 1948	$ 845.00
Disbursements (Taxes)	85.44

All Gansett lots having been sold, cash balance of $522.20 representing net profit on sale of last five lots was transferred to Current Income account, and Gansett account was closed.

VIII. Agency Accounts

A. Cancer Research Account

(U. S. Public Health Service Project under direction of Dr. Robert Chambers)

Jan. 1, 1948, Balance on Hand		$18,721.19
Disbursements:		
Payments for Salaries, Laboratory Space, Apparatus and Supplies	$18,719.96	
Balance Remitted to the United States Treasury	1.23	18,721.19
		– 0 –

EXHIBIT A

Marine Biological Laboratory Balance Sheet, Dec. 31, 1948

(From Auditors' Report)

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 961,729.66	
Securities and Cash in Minor Funds		18,135.52	$ 979,865.18
Plant Assets:			
Land	$ 110,626.38		
Buildings	1,345,956.86		
Equipment	238,252.31		
Library	374,880.39		
	$2,069,715.94		
Less Reserve for Depreciation	747,963.45	$1,321,752.49	
Book Fund, Securities and Cash		11,416.15	$1,333,168.64
Current Assets:			
Cash		$ 15,257.21	
Mortgage Note Receivable		2,350.00	
Accounts Receivable		24,420.03	
Inventories:			
Supply Department	$ 49,974.46		
"Biological Bulletin"	16,195.99	66,170.45	
Investments:			
Devil's Lane Property	43,401.43		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Securities	35,020.00		
Retirement Fund	18,195.08	109,316.51	
Prepaid Insurance		5,959.57	
Items in Suspense (Debits)		2,092.72	$ 225,566.49
			$2,538,600.31

Liabilities

Endowment Funds:			
Endowment Funds	$ 960,016.27		
Reserve for Amortization	1,713.39	$ 961,729.66	
Minor Funds		18,135.52	$ 979,865.18
Plant Funds:			
Mortgage Notes Payable		$ 10,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	150,604.60	1,323,168.64	$1,333,168.64
Current Liabilities and Surplus:			
Accounts Payable		$ 13,552.25	
Items in Suspense (Credits)		2,582.07	
Current Surplus		209,432.17	$ 225,566.49
			$2,538,600.31

Respectfully submitted,

Donald M. Brodie,
Treasurer

V. REPORT OF THE ACTING LIBRARIAN

1948

In 1948 the Library staff carried on the necessary routine activities and continued with projects already in progress.

The sum of $11,500 was appropriated to the Library, plus $3000 from the Woods Hole Oceanographic Institution, a contribution toward the salaries of the staff. For a detailed account of the expenditures of the budget, $13,077.73, reference may be made to the Treasurer's report. The sum of $800 was provided by the Woods Hole Oceanographic Institution for library acquisitions.

During the year, 1209 (55 new) current journals were received. Of these, 329 (12 new) were Marine Biological Laboratory subscriptions, 510 (14 new) were exchanges, and 168 (3 new) were gifts; 51 (4 new) were Woods Hole Oceanographic Institution subscriptions, 137 (17 new) were exchanges, and 14 (5 new) were gifts. Due to the great irregularity in current receipts of German and Russian journals, most of these titles were omitted in this tabulation.

The Marine Biological Laboratory purchased 78 books, received 9 complimentary copies from authors, 43 gifts from the publishing firms, 152 books from the E. L. Mark Library, and 23 miscellaneous donations. The Woods Hole Oceanographic Institution purchased 32 books. These made a total of 337 titles acquired.

There were 27 back sets completed: 18 by purchase (5 Woods Hole Oceanographic Institution), 6 by exchange (3 Woods Hole Oceanographic Institution), and 3 by gift (2 Woods Hole Oceanographic Institution); 55 were partially completed: 39 by purchase (9 Woods Hole Oceanographic Institution), 1 by exchange (Woods Hole Oceanographic Institution), and 15 by gift.

The reprint additions to the Library numbered 11,310. Of these, 1385 were of current issue and the others were of earlier dates. Of the 24,690 papers acquired through the collections of Drs. Ulrich Dahlgren, Alfred C. Redfield, Frank R. Lillie, and a gift from the Boston University School of Medicine, 4642 were found to be reprints not already appearing in the Library's collection.

A sum of $2989.85 from the Carnegie Corporation of New York Fund was spent for 6 books, 9 completed back sets, 16 partially completed back sets, and the binding of 337 volumes.

There were 86 microfilm orders filled during the year. Libraries and individuals have been encouraged to use this service in preference to requesting volumes on inter-library loan. In spite of this effort, 94 volumes were sent out on loan, and 51 were borrowed for the investigators.

Several valuable gifts were received in 1948. The most outstanding of these was the collection of Dr. Frank R. Lillie's reprints. Dr. Lillie's thought in requesting that this Library inherit his collection was one of the many kindnesses shown throughout the years of his association with this Laboratory. Grateful acknowledgment is also made to the Bermuda Biological Station for Research, Inc., for the gift of the E. L. Mark Library of books and reprints. Time has not permitted the complete assorting of this huge amount of material, but 152 books and 1600 reprints have been added to the shelves. Among these were several very valuable books by Louis Agassiz. The Library is also indebted to the American

Academy of Arts and Sciences for a four-volume set of Count Rumford's works.

The Library contained at the end of 1948, 57,548 bound volumes and 160,528 reprints.

Respectfully submitted,
DEBORAH LAWRENCE
Acting Librarian

VI. THE REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY

Gentlemen:

I submit herewith a report of the sixty-first session of the Marine Biological Laboratory for the year 1948.

During the past year our physical plant has undergone many changes, chief of which are the renovation of Old Main; the purchase of the Elliott House; the purchase of the Dolphin; and the development of a laboratory for work with radioactive isotopes.

1. *Renovation of Old Main*

The generous gift of $150,000 made by the Rockefeller Foundation for the renovation of Old Main has enabled us to make necessary and long desired changes in that building. Preliminary plans for the work were prepared by the firm of Coolidge, Shepley, Bulfinch, and Abbott in consultation with the Building Committee consisting of Mr. Claff, Chairman, and Drs. Marsland, Parpart, and Packard. This Committee was later enlarged to include Messrs. MacNaught, Smith, Robert Kahler, and Pierce of the Laboratory Staff. Because of the high cost of construction not all of the changes desired by the Committee could be made. However, with the available funds, the building, which is structurally sound, can be put into excellent condition.

It was decided to construct a basement under the entire building during the Spring of 1949, and to finish the renovation of the upper floors after the close of the summer session. By this arrangement, the classes can be held as usual, without any interruption. The Spring operations are in charge of the Sawyer Construction Company which built the new wing of the Library. The basement contains ten research rooms, two general laboratories, all equipped with sea water tables, and other necessary facilities, three dark rooms with sea water tables, a cold room (to be completed later), and service rooms. Between the wings of the building a sunken court provides light for the rooms opening on to it, and space for two large fish pools. The old plumbing, for many years in deplorable condition, has been completely replaced in the basement, and will be greatly improved elsewhere after the close of the summer season. Additions to the northwest and southwest corners of the building give extra space in the basement and on the first and second floors. The total area of the basement plus these newly constructed rooms is about equal to one floor of the Crane Building. Thanks to the Rockefeller Foundation we shall have a laboratory which will adequately accommodate the classes and the instructors for many years.

Some of our workers have doubted the wisdom of spending $150,000 on an old building, while others are pleased that Old Main, where so much important biological research has been carried on during the past sixty years, will be used by new generations of students. While a completely new structure would be desirable, there seemed to be no prospect of obtaining funds for the construction and equipment. On the other hand, the old building is well worth preserving. Mr. Sawyer, under whose direction the renovation has been made, writes that "The existing walls, floors and roof construction in Old Main are in excellent condition, although certain parts of the wall and roof shingling must be renewed in the comparatively near future. In our opinion, you have done the economical thing in making use of as much of Old Main as is possible."

2. *The Elliott House*

As a result of the purchase of the Elliott House the Laboratory now owns all the property on both sides of Center Street. This house, which will accommodate about twenty people, has been improved by the addition of an upstairs bathroom, and other changes.

3. *The Dolphin*

The purchase of a new boat, in addition to the *Limulus,* was mentioned in the last Annual Report. The new craft, the *Dolphin,* was in service during the summer of 1948, taking the classes on field trips and making collections of living material. It accommodates seventy passengers, is seaworthy and fast, and is a welcome addition to the fleet. Its gasoline engine has now been replaced by a Diesel motor acquired from the War Assets Administration and installed by our machinist, Mr. Harlow.

4. *The Radiation Laboratory*

Under the direction of Dr. Failla, the Radiation Laboratory has been greatly expanded. The American Cancer Society last year contributed $4,300 for the purchase of measuring instruments and other apparatus, and for equipment needed to safeguard investigators using radio-active substances. A "hot laboratory" where these materials can be transferred is now furnished with special tables, cabinets, and a hood.

This new field of research has attracted many workers. During the summer of 1948 fifteen investigators were using radio-active isotopes, among them being four Fellows supported by funds granted by the Atomic Energy Commission.

To carry on this type of research successfully, investigators require special training. For this reason Dr. Failla arranged a course of lectures given by well known specialists. Dr. Paul Aebersold, Chief of the Isotopes Division at Oak Ridge, took an active part in the course, and was available for advice on technical problems.

5. *Winter Research*

During a large part of the year the Institute of Muscle Research, under the direction of Dr. Szent-Gyorgyi has carried on research here. The number of investigators has grown until now there are eight at work. The laboratory of Experimental Cell Research, directed by Dr. Chambers, occupied rooms during the winter of 1947–48, but has now moved to New York City.

6. *Gifts*

The Rockefeller Foundation, in addition to its gift of $150,000 for the renovation of Old Main, has also granted for the general support of the Laboratory the sum of $100,000, available during the next five years at the rate of not more than $25,000 per year. This is most welcome. for we stand in need of funds to replace old equipment, especially in the Apparatus and Chemical Departments, and to purchase other apparatus required for research along lines not heretofore followed at this Laboratory. This gift, however, is not restricted to such purposes and can be used for other laboratory needs.

In his will, Dr. F. R. Lillie bequeathed to the Laboratory 500 shares of Crane Company stock, the book value of which is $17,250.

Other gifts have been received from

The Schwarzhaupt Foundation towards the purchase of a new boat	$1,000
Dr. W. D. Curtis, for a bandsaw in the Carpenter Shop	$225
Mr. Leo H. Spivack	$200
The M. B. L. Associates	$1,320
The American Cancer Society to purchase apparatus to be used in the Laboratory of Experimental Cell Research and for	$5,000
equipment and technical help in the Radiation Laboratory	$4,300

7. *Changes in Personnel*

Dr. E. P. Little, manager of the Apparatus Department since 1942, and of the Chemical Department during the war years, has resigned in order to carry on research at the Computation Laboratory of Harvard University.

During his incumbency he greatly enlarged the Apparatus Department and increased its efficiency. We are glad to retain him as a Consultant. The new manager is Mr. Robert Mills who has been associated with Dr. Little for some time. Mr. Gail Cavanaugh, head of the Science Department at the Falmouth High School, has been appointed manager of the Chemical Department.

We shall miss "Colonel" Wamsley who died in the spring of 1949. Coming here in 1892 as a student from Brown University he became a member of the Corporation. He was connected with the Charleston, S. C. Museum for many years, and served as a superintendent of one of the city schools. During the summers he was a special collector in the Supply Department.

8. *Election of Trustees*

At the meeting of the Corporation, August 10, 1948, the following trustees were elected.

Class of 1952

E. S. G. Barron
D. W. Bronk
G. Failla
C. O'D. Iselin
R. T. Kempton
C. W. Metz
Wm. Randolph Taylor
George Wald

Albert Tyler was elected to fill the vacancy caused by the death of S. C. Brooks of the Class of 1949.

H. H. Plough was elected in place of G. H. A. Clowes of the Class of 1951, who was made Trustee Emeritus.

9. *There are appended as parts of this report:*

1. The Staff
2. Investigators and Students
3. The Lalor Fellows
4. The Atomic Energy Commission Fellows
5. Tabular View of Attendance
6. Subscribing and Cooperating Institutions
7. Evening Lectures
8. Shorter Scientific Papers Presented at the Seminar
9. Members of the Corporation

Respectfully submitted,
CHARLES PACKARD,
Director

1. THE STAFF, 1948

CHARLES PACKARD, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

SENIOR STAFF OF INVESTIGATION

E. G. CONKLIN, Professor of Zoology, Emeritus, Princeton University.
RALPH S. LILLIE, Professor of General Physiology, Emeritus, The University of Chicago.
A. P. MATHEWS, Professor of Biochemistry, Emeritus, University of Cincinnati.
G. H. PARKER, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. CONSULTANTS

LIBBIE H. HYMAN, American Museum of Natural History.
A. C. REDFIELD, Woods Hole Oceanographic Institution.

II. INSTRUCTORS

F. A. BROWN, Professor of Zoology, Northwestern University, in charge of course.
W. D. BURBANCK, Professor of Biology, Drury College.
C. G. GOODCHILD, Professor of Biology, S.W. Missouri State College.
L. H. KLEINHOLZ, Associate Professor, Reed College.
JOHN H. LOCHHEAD, Assistant Professor of Zoology, University of Vermont.
MADELENE E. PIERCE, Associate Professor of Zoology, Vassar College.
W. M. REID, Professor of Biology, Monmouth College.
T. H. WATERMAN, Assistant Professor in Biology, Yale University.

III. LABORATORY ASSISTANTS

R. S. HOWARD, University of Miami.
MARIE WILSON, Northwestern University.

EMBRYOLOGY

I. Consultants

H. B. Goodrich, Professor of Biology, Wesleyan University.
Albert Tyler, Associate Professor of Embryology, California Institute of Technology.

II. Instructors

Donald P. Costello, Prfoessor of Zoology, University of North Carolina, in charge of course.
Arthur L. Colwin, Assistant Professor of Zoology, Queens College.
Howard L. Hamilton, Associate Professor of Zoology, Iowa State College.
Charles B. Metz, Assistant Professor of Zoology, Yale University.

III. Research Assistant

Marjorie Hopkins Fox, University of California.

IV. Laboratory Assistant

Helen A. Padykula, Mount Holyoke College.

Marine Biological Laboratory Library Woods Hole, Mass.

PHYSIOLOGY

I. Consultants

Eric G. Ball, Professor of Biochemistry, Harvard University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.
Otto Loewi, Professor of Pharmacology, New York University, School of Medicine.
Arthur K. Parpart, Professor of Biology, Princeton University.

II. Instructors

E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago, in charge of course.
M. J. Kopac, Associate Professor of Biology, New York University.
John F. Muntz, Assistant Professor of Biochemistry, Western Reserve University Medical School.
Robert F. Pitts, Professor of Physiology, Syracuse University, College of Medicine.
H. Burr Steinbach, Professor of Zoology, University of Minnesota.
George Wald, Professor of Biology, Harvard University.
Dorothy Wrinch, Lecturer, Smith College.

BOTANY

I. Consultants

P. R. Burkholder, Eaton Professor of Botany, Yale University.
W. R. Taylor, University of Michigan.

II. Instructors

Maxwell S. Doty, Assistant Professor of Botany, Northwestern University. In Charge of Course.
H. C. Bold, Vanderbilt University.
R. D. Northcraft, Rutgers University.

III. Research Assistants

Justine Garnic, Carnegie Institute of Technology.
Leonard E. Spiegel, Drew University.

IV. Laboratory Assistant

Remi J. Cadoret, Harvard University.

V. Lecturers

J. B. Lackey, Philadelphia, Pa.
R. D. Wood, Rhode Island State College.

VI. Field Consultant and Collector

Edwin T. Moul, University of Pennsylvania.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Deborah Lawrence, Acting Librarian
Margaret P. McInnis
Mary A. Rohan
Jean Goodfellow

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham
Dorothy LeFevre
Robert Mills

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist
Ruth Crowell
Marcia McLaughlin
M. B. Gray
A. M. Hilton
W. E. Kahler
W. S. Landers
G. Lehy
R. E. Tonks
F. N. Whitman

GENERAL OFFICE

F. M. MacNaught, Business Manager
Homer P. Smith, Assistant Business Manager
Polly L. Crowell
Mrs. Lila S. Myers

GENERAL MAINTENANCE

R. W. Kahler, Manager

Robert Adams
R. Gunning
J. H. Head
G. A. Kahler
A. Neal
G. T. Nichelson, Jr.
A. J. Pierce
T. E. Tawell
Seaver R. Harlow

THE GEORGE M. GRAY MUSEUM

2. INVESTIGATORS AND STUDENTS

Independent Investigators, 1948

ABELSON, PHILIP H., Staff Member, Carnegie Institute of Washington.
ABRAMS, RICHARD, Assistant Professor, University of Chicago.
AEBERSOLD, PAUL C., Chief, Isotopes Branch, Atomic Energy Commission.
AGERSBORG, H. P. K., Professor of Histology. Des Moines Still College.
ALLEN, M. JEAN, Instructor in Biology, Mather College.
AMBERSON, WILLIAM R., Professor of Physiology, University of Maryland Medical School.
ARDAO, MARIA ISABEL, University of Montevideo.
ARMAGHAN, VERONICA, New York City.
ARMSTRONG, PHILIP B., Professor of Anatomy. College of Medicine, Syracuse University.
AUGUSTINSSON, KLAS-BERTIL, University of Stockholm.
BAKER, GLADYS E., Associate Professor of Plant Science, Vassar College.
BALDWIN, ERNEST H. F., University Lecturer in Biochemistry, Cambridge, England.
BALL, ERIC G., Professor of Biological Chemistry, Harvard Medical School.
BARRON, E. S. GUZMAN, Associate Professor of Biochemistry, University of Chicago.
BERGER, CHARLES A., Director, Biological Laboratory, Fordham University.
BLISS, ALFRED F., Assistant Professor of Physiology, Tufts Medical School.
BLUM, HAROLD F., Physiologist, National Cancer Institute.
BONNER, JOHN T., Assistant Professor of Biology, Princeton University.
BOWERS, JOHN Z., Assistant and Director and Chief, U. S. Atomic Energy Commission.
BOYER, DONALD D., Instructor in Biology, Union College.
BRIDGMAN, ANNA J., Professor of Biology, Limestone College.
BROOKS, MATILDA, Professor of Zoology, University of California.
BROWN, FRANK A., JR., Professor of Zoology, Northwestern University.
BURBANCK, WILLIAM D., Professor of Biology. Drury College.
BUTLER, ELMER G., Professor of Zoology, Princeton University.
CAMERON, GLADYS, Research Associate, New York City.
CANNAN, R. KEITH, Professor of Chemistry, New York University College of Medicine.
CHAMBERS, ROBERT, Chief, Laboratory of Experimental Cell Research.
CHANCE, BRITTON, Assistant Professor of Biophysics, Johnson Research Foundation.
CHASE, AURIN M., Associate Professor of Biology, Princeton University.
CHENEY, RALPH H., Associate Professor of Biology, Brooklyn College.
CHRYSTALL, FRIEDA L., Teacher of Biology, Julia Richman High School.
CHU, HAO-JAN, Graduate Assistant in Botany, Northwestern University.
CLAFF, C. LLOYD, Research Fellow in Surgery, Harvard Medical School.
CLARK, ARNOLD M., Instructor in Biology, University of Delaware.
CLARK, ELIOT R., Professor of Anatomy, University of Pennsylvania School of Medicine.
CLARK, LEONARD B., Professor of Biology, Union College.
CLAUDE, ALBERT, Associate Member, The Rockefeller Institute.
CLEMENT, A. C., Professor of Biology, College of Charleston.
CLOWES, G. H. A., Research Director Emeritus, Lilly Research Laboratories.
COHEN, ISADORE, Assistant Professor, American International College.
COLE, KENNETH S., Professor of Biophysics, University of Chicago.
COLWIN, ARTHUR L., Assistant Professor of Biology, Queens College.
CONKLIN, EDWIN G., Professor of Biology Emeritus, Princeton University.
COOPER, KENNETH W., Associate Professor of Biology, Princeton University.
COPELAND, E. EUGENE, Assistant Professor of Biology, Brown University.
CORNMAN, IVOR, Research Fellow, Sloan-Kettering Institute.
COSTELLO, DONALD P., Professor of Zoology, University of North Carolina.
COYLE, ELIZABETH E., Assistant Professor of Biology, College of Wooster.
CROWELL, SEARS, Associate Professor of Zoology, Miami University.
DENT, J. N., Assistant Professor, University of Pittsburgh.
DILLER, IRENE C., Research Cytologist, Institute for Cancer Research, Lankinau Hospital.

Diller, William F., Assistant Professor of Zoology, University of Pennsylvania.
Doty, Maxwell S., Assistant Professor of Botany, Northwestern University.
Duryee, William R., Research Associate, Carnegie Institution of Washington.
Edgerley, Robert H., Research Scientist. Columbia University.
Eichel, Bertram, Research Associate, New York University College of Dentistry.
Evans, Titus C., Research Professor of Radiology, State University of Iowa.
Fahey, Elizabeth M., Teacher, Taunton High School.
Failla, G., Professor of Radiology, Columbia University.
Flood, Veronica M., Junior Scientist, Argonne National Laboratories.
Frenkel, Albert W., Assistant Professor of Botany, University of Minnesota.
Froehlich, Alfred, Associate, The May Institute for Medical Research.
Gaffron, Hans, Associate Professor of Biochemistry, University of Chicago.
Gerard, Pol, Professor of Pathology, The Belgian American Educational Foundation.
Glaser, O. C., Professor of Biology, Amherst College.
Goldstein, Avram, Instructor in Pharmacology, Harvard University Medical School.
Goodchild, C. G., Professor of Biology, Missouri State College.
Gould, Harley N., Professor of Biology. Tulane University, Newcomb College.
Grand, C. G., Research Associate, New York University.
Gray, Irving M., Professor of Zoology, Duke University.
Greenberg, G. Robert, Senior Instructor in Biochemistry, Western Reserve University.
Gregg, John R., Assistant Professor of Zoology, Columbia University.
Grosch, Daniel S., Assistant Professor of Zoology, North Carolina State College.
Grundfest, Harry, Assistant Professor of Neurology, Columbia University.
Hamilton, Howard L., Associate Professor of Zoology, Iowa State College.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Harvey, Ethel Browne, Independent Investigator, Princeton University.
Haywood, Charlotte, Professor of Physiology, Mount Holyoke College.
Heidenthal, Gertrude, Assistant Professor of Biology, Russell Sage College.
Heilbrunn, L. V., Professor of Zoology, University of Pennsylvania.
Hestrin, Shlomo, Research Assistant, College of Physicians and Surgeons.
Hopkins, Hoyt S., Associate Professor of Physiology, New York University College of Dentistry.
Hsiao, Sidney Co., Guest Professor of Biology, Osborn Zoological Laboratory.
Hunter, Francis R., Associate Professor of Zoology, University of Oklahoma.
Hutchens, John O., Chairman, Department of Physiology, University of Chicago.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Janney, Clinton D., Research Assistant Professor of Physiology, State University of Iowa.
Jenkins, George B., Professor of Anatomy Emeritus, George Washington University.
Kabat, Elvin A., Assistant Professor of Bacteriology, College of Physicians and Surgeons.
Karush, Fred, Fellow, New York University College of Medicine.
Keller, Rudolph, Director of Biochemical Research, Madison Foundation.
Kempton, Rudolf T., Professor of Zoology. Vassar College.
Kisch, Bruno, Professor, Yeshiva University.
Kitchin, Irwin C., Associate Professor of Biology, University of Georgia.
Kleinholz, Lewis H., Associate Professor of Biology, Reed College.
Klotz, Irving M., Associate Professor of Chemistry, Northwestern University.
Kopac, M. J., Associate Professor of Biology. New York University.
Krahl, Maurice E., Assistant Professor of Pharmacology, Washington University.
Lazarow, Arnold, Assistant Professor of Anatomy, Western Reserve University.
LeFevre, Paul G., Assistant Professor of Physiology, University of Vermont.
Lein, Joseph, Instructor in Biology, Syracuse University.
Levy, Milton, Associate Professor of Chemistry, New York University College of Medicine.
Libet, Benjamin, Assistant Professor of Physiology, University of Chicago.
Lillie, Ralph S., Professor of Physiology Emeritus, University of Chicago.
Liu, Chien Kang, Research Fellow, Laboratory of Experimental Cell Research.
Lochhead, John H., Assistant Professor of Zoology, University of Vermont.
Lovelace, Roberta, Adjunct Professor of Biology, University of South Carolina.
Lucke, Balduin, Professor of Pathology, University of Pennsylvania.

Marshak, Alfred, Research Associate, New York University Medical College.
Marsland, Douglas A., Professor of Biology, New York University, Washington Square College.
Mazia, Daniel, Professor of Zoology, University of Missouri.
Metz, Charles B., Assistant Professor of Zoology, Yale University.
Michalski, Joseph V., Assistant Professor of Anatomy, Emory University.
Miller, James A., Associate Professor of Anatomy, Emory University.
Muntz, John A., Assistant Professor, Western Reserve University.
Nachmansohn, David, Assistant Professor of Neurology, College of Physicians and Surgeons.
Nauss, Mrs. Ralph, 1303 York Avenue, New York City.
Nelson, Leonard, Teaching Assistant in Zoology, University of Minnesota.
Newfang, Dorothy M., Assistant Professor of Botany, Elmira College.
Northrop, John H., Head of Department of General Physiology, Rockefeller Institute.
Nutting, William B., Instructor in Zoology, University of Massachusetts.
O'Brien, John A., Assistant Professor of Biology, Catholic University of America.
Olson, Magnus, Assistant Professor of Zoology, University of Minnesota.
Oster, Robert H., Professor of Physiology, University of Maryland.
Osterhout, W. J. V., Member Emeritus, Rockefeller Institute.
Palay, Sanford L., Visiting Investigator, Rockefeller Institute.
Parmenter, C. L., Professor of Zoology, University of Pennsylvania.
Parpart, Arthur K., Professor of Biology, Princeton University.
Parshley, H. M., Professor of Zoology, Smith College.
Pasteels, Jean J., Professor of Anatomy, University of Brussels.
Persky, Harold, Director of Research, Michael Reese Hospital.
Pick, Joseph, Associate Professor of Anatomy, New York University College of Medicine.
Pierce, Madelene E., Associate Professor of Zoology, Vassar College.
Pitts, Robert F., Professor of Physiology, Syracuse University College of Medicine.
Pocock, Mary A., Senior Lecturer in Botany, Rhodes University, South Africa.
Prat, Henri, Professor, University of Montreal.
Price, Winston H., Special Investigator, Rockefeller Institute.
Prosser, C. Ladd, Associate Professor of Zoology, University of Illinois.
Raut, Caroline, Assistant Professor, Southern Illinois University.
Reid, W. Malcolm, Professor of Biology, Monmouth College, Monmouth, Illinois.
Rose, S. Meryl, Associate Professor of Zoology, Smith College.
Rossi, Harold H., Physicist, Department of Radiology, Columbia University.
Rudzinski, Marie A., Instructor, New York University, Washington Square College.
Rugh, Roberts, Associate Professor of Biology, New York University.
Schaeffer, A. A., Professor of Biology, Temple University.
Schmidt, Gerhard, Senior Research Associate, Tufts College Medical School.
Scott, Allan C., Associate Professor of Biology, Union College.
Scott, Sister Florence Marie, Professor of Biology, Seton Hill College.
Scott, George T., Assistant Professor of Biology, Oberlin College.
Shanes, Abraham M., Associate Professor of Physiology, Georgetown University School of Medicine.
Sichel, F. J., Professor of Physiology, University of Vermont.
Slifer, Eleanor H., Assistant Professor of Zoology, State University of Iowa.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Stekler, Burton L., Student, New York University College of Medicine.
Steinbach, H. B., Professor of Zoology, University of Minnesota.
Stewart, Dorothy R., Associate Professor of Zoology and Physiology, Rockford College.
Straus, William L., Jr., Associate Professor of Anatomy, Johns Hopkins University.
Szent-Györgyi, A. E., Szent-Györgyi Research Foundation.
Tahmisian, Theodore N., Associate Scientist, Argonne National Laboratories.
Taylor, Wm. Randolph, Professor of Botany, University of Michigan.
TeWinkel, Lois, Associate Professor of Zoology, Smith College.
Ting, Te-Pang, Eli Lilly Fellow, Institute of Radiobiology, University of Chicago.
Tracy, H. C., Professor of Anatomy, University of Kansas.
Trinkaus, J. Philip, Instructor in Zoology, Osborn Zoological Laboratory.

Tung, Ti-Chow, Special Fellow, Rockefeller Foundation, Osborn Zoological Laboratory.
Tyler, Albert, Associate Professor of Embryology, California Institute of Technology.
Tyler, David B., Research Associate, Carnegie Institute of Washington.
Villee, Claude A., Associate in Biological Chemistry, Harvard Medical School.
Wainio, Walter W., Assistant Professor of Physiology, New York University College of Dentistry.
Wald, George, Professor of Biology, Harvard University.
Walker, Ruth I., Professor of Botany, University of Wisconsin.
Warner, Robert C., Assistant Professor of Chemistry, New York University College of Medicine.
Waterman, Talbot H., Assistant Professor of Zoology, Yale University.
White, Elizabeth L., Instructor in Zoology, Washington University.
Whiting, Anna R., Guest Investigator, University of Pennsylvania.
Whiting, P. W., Professor of Zoology, University of Pennsylvania.
Wichterman, Ralph, Associate Professor, Temple University.
Woodward, Arthur A., Jr., Instructor in Biology, Brown University.
Wrinch, Dorothy, Lecturer in Physics, Smith College.
Yudkin, Warren H., Graduate Student, Yale University.
Zinn, Donald J., Instructor in Zoology, Rhode Island State College.
Zorzoli, Anita, Instructor in Physiology, Washington University School of Dentistry.

Beginning Investigators, 1948

Anagnostis, Irene P., Student, New York University.
Alscher, Ruth P., Instructor in Biology, Manhattanville College.
Boyle, E. Marie, Science Teacher, Baldwin School.
Bulloch, Jane Ann, Student, University of Oklahoma.
Cohen, Arthur I., Student, University of Minnesota.
Cooperstein, Sherwin J., Student, New York University College of Dentistry.
Coret, Irving A., Research Fellow, University of Pennsylvania.
Eichel, Herbert J., Graduate Student, New York University.
Essner, Edward S., Graduate Student, University of Pennsylvania.
Fitch, Naomi, Assistant in Zoology, Columbia University.
Gagnon, Andre, Research Assistant, University of Pennsylvania.
Gasvoda, Betty M., Junior Scientist, Argonne National Laboratories.
Goodkind, M. Jay, Student, Princeton University.
Goreau, Thomas F., Medical Student, University of Pennsylvania.
Green, James W., Research Associate, Princeton University.
Gregg, James H., Graduate Student, Princeton University.
Harding, Clifford V., Jr., Graduate Student, University of Pennsylvania.
Hay, Elizabeth D., Student, Smith College.
Hirschhorn, Henry A., Student, New York University College of Dentistry.
Hodgson, Edward S., Jr., Junior Instructor, Johns Hopkins University.
Hoffman, Joseph F., Student, University of Oklahoma.
Honegger, C. M., Instructor, Temple University.
Hopkins, Amos L., Jr., Graduate Student, University of Pennsylvania.
Jacobson, Julius H., Graduate Student, University of Pennsylvania.
Jones, Gwen Maxine, Teaching Assistant, Northwestern University.
Kelly, John W., Graduate Student, University of Pennsylvania.
Kozam, George, Instructor, New York University College of Dentistry.
Martin, Barbara Adele, Assistant in Zoology, Barnard College.
Moskovic, Samuel, Teaching Fellow, Graduate School, New York University.
Nardone, Roland M., Laboratory Assistant, Fordham University.
Nelson, Thomas Clifford, Lecturer in Biophysics, Columbia University.
Padykula, Helen A., Instructor, Wellesley College.
Ray, David T., Graduate Student, University of Pennsylvania.
Rieser, Peter, Assistant Instructor, University of Pennsylvania.
Rosenbaum, Joan, Student, Columbia University.
Rosenbluth, Raja, Graduate Student, Columbia University.
Rossetti, Fiammetta, Student, University of Chicago.

Rothberg, Harvey, Jr., Undergraduate, Princeton University.
Seaman, Gerald R., Graduate Assistant in Biology, Fordham University.
Susca, Louis A., Instructor, Fordham University.
Sze, L. C., Research Assistant, Columbia University.
Wilson, Walter L., Research Assistant, University of Pennsylvania.

Research Assistants, 1948

Baily, Norman A., Research Scientist, Columbia University.
Benson, Eleanore, Research Assistant, University of Missouri.
Berman, Jack H., Fellow in Anatomy, Western Reserve University.
Blumenthal, Gertrude, Research Associate, University of Missouri.
Clark, Grace, Laboratory Technician, Columbia University.
Cooper, Octavia, Research Assistant, Harvard University Medical School.
Crane, Robert K., Chemist, Eli Lilly and Company.
Curtis, Paul, Student, Bethany College.
Eggers, Annette, Acting Instructor, Stanford University.
Fass, Gerome S., Research Technician, Rockefeller Institute for Medical Research.
Foley, Mary T., Research Assistant, Yale University.
Fusco, Edna Marie, Research Assistant, Yale University.
Garnic, Justine, Graduate, Carnegie Institute of Technology.
Gordon, Marcia, Research Assistant, Harvard Medical School.
Henley, Catherine, Research Assistant, University of North Carolina.
Hickson, Anna Keltch, Research Chemist, Eli Lilly and Company.
Himmelfarb, Sylvia, Research Assistant, University of Maryland Medical School.
Howard, Robert S., Graduate Assistant, University of Miami.
Jacob, Miriam, Technician, Rockefeller Institute.
Keeney, Belle C., Stanford University.
Kimberly, Paul E., Associate Professor, Des Moines Still College.
Kirk, Marjorie J., Student, Harvard Medical School.
LeFevre, Marian E., Graduate Student, University of Vermont.
Lemm, Frances J., Research Assistant, Western Reserve University.
Lesse, Henry, Medical Student, Jefferson Medical College.
Litovchick, Mortimer, Research Technician, Rockefeller Institute.
Love, Lois H., Instructor, University of Pennsylvania.
Love, Warner E., Assistant Instructor, University of Pennsylvania.
Lowens, Mary D., Research Assistant, Harvard Medical School.
Metcalf, John S., Jr., Medical Student, University of Maryland School of Medicine.
Mitchell, Constance, Instructor, University of Delaware.
Nach, Lucille, Research Assistant, Western Reserve University.
Passano, Leonard M., III, Graduate Student, Yale University.
Podolsky, Betty, Research Assistant, University of Chicago.
Raskind, Josephine B., Bryn Mawr College.
Rawley, June, Student, University of Oklahoma.
Rich, Alexander, Research Assistant, Harvard Medical School.
Roth, Alexander, Research Assistant, University of Kansas.
Rothenberg, Mortimer A., Research Assistant, Columbia University.
Sandeen, Muriel I., Teaching Assistant, Northwestern University.
Seaman, Arlene R., Assistant, Cornell University.
Shedd, Donald H., Dartmouth College.
Slattery, Leo F., Research Assistant, University of Chicago.
Spiegel, Leonard E., Graduate Assistant in Botany, Northwestern University.
Van Hoesen, Drusilla, Research Associate, University of Pennsylvania.
Volkman, Alvin, Student, University of Buffalo.
Walters, C. Patricia, Chemist Assistant, Eli Lilly and Company.
Webb, H. Marguerite, Teaching Assistant, Northwestern University.
Wetstone, Howard J., Research Assistant, Smith College.
Wilson, Marie, Teaching Assistant, Northwestern University.
Winblad, James N., Research Assistant, University of Kansas Medical School.

Library Readers, 1948

Abell, Richard G., Resident in Psychiatry, College of Physicians and Surgeons.
Adler, Francis H., Professor of Ophthalmology, University of Pennsylvania.
Adlersberg, David, Adjunct Physician, Mt. Sinai Hospital.
Anderson, Rubert S., Assistant Professor of Physiology, University of Maryland Medical School.
Bartlett, James H., Professor of Physics, University of Illinois.
Berman, Evelyn M., Teacher, Montreal Protestant.
Blair, John H., Assistant Professor of Physiology, University of Massachusetts.
Boroff, Daniel A., Research Bacteriologist.
Curtis, W. C., Dean and Professor of Zoology Emeritus, University of Missouri.
Dische, Zacharias, Assistant Professor of Biochemistry, College of Physicians and Surgeons.
Ehrlich, Miriam, Graduate Student, Yale University.
Foley, Joseph B., Graduate Student, Yale University.
Freund, Jules, Chief, Public Health Research Institute of the City of New York.
Gates, R. Ruggles, Research Fellow in Biology, Harvard University.
Grant, Madeleine P., Member of Science Faculty, Sarah Lawrence College.
Gudernatsch, Frederick, Retired Visiting Professor, New York University.
Gurewich, Vladimir, Assistant Visiting Physician, Bellevue Hospital.
Hanke, Harriett, Teaching Fellow in Biology, New York University.
Hensley, Ruth A., Graduate Assistant in Zoology, University of Missouri.
Hess, Eckhard H., The University of Chicago.
Hill, Ruth F., Assistant Physicist, Sloan-Kettering Institute.
Hutchings, Lois M., Instructor, Drew University.
Kaplan, Ann E., Graduate Assistant, Mt. Holyoke College.
Kaupe, Walter, Research Assistant, Massachusetts Institute of Technology.
Kauzmann, Walter, Assistant Professor of Chemistry, Princeton University.
Keane, John Francis, Fordham University.
Keeffe, Mary M., Assistant Professor of Biology, College of St. Thomas.
Krasnow, Frances, Head of Department of Research, The Guggenheim Dental Foundation.
Leighton, Joseph, Massachusetts General Hospital.
Leikind, Morris C., Head of Biology and Medicine Unit, Library of Congress.
Lisi, Alfred G., Instructor, Department of Pharmacology, Jefferson Medical College.
Loewi, Otto, Research Professor, New York University College of Medicine.
McDonald, Sister Elizabeth Seton, Professor of Biology, Mt. St. Joseph College.
Martin, Arthur W., Associate Professor of Physiology, University of Washington.
Mathews, Albert P., Carnegie Professor of Biochemistry Emeritus, University of Chicago.
Meyerhof, Otto, Research Professor, University of Pennsylvania.
Miller, Elizabeth M., Technician, Rockefeller Institute.
Schiller, Paul H., Research Associate, Yerkes Laboratories.
Schuh, Joseph E., Graduate Student, Columbia University.
Shapiro, Herbert, Physiologist, National Institute of Health.
Shwartzman, Gregory, Head of Department of Bacteriology, Mt. Sinai Hospital.
Smith, Sydney, Research Associate, University of Rochester.
Stauffer, Robert C., Assistant Professor of History of Science, University of Wisconsin.
Stern, Kurt G., Adjunct Professor of Biochemistry, Polytechnic Institute.
Sulkin, S. Edward, Professor and Chairman, Southwestern Medical College.
Tagnon, Henry J., Associate Member, Sloan-Kettering Institute.
Therman, Olaf, Director of Laboratories, Pennsylvania Hospital.
Tutelman, Harriet, Graduate Student, Johns Hopkins University.
Willier, B. H., Professor of Zoology, Johns Hopkins University.
Yntema, Chester L., Associate Professor of Anatomy, Syracuse University Medical School.

Students, 1948

BOTANY

Abbott, Robinson S., Student, Bucknell University.
Battley, Edwin H., Student, Harvard College.

Bernatowicz, Albert J., Student, Clark University.
Cowen, Naomi, Student, Cornell University.
Diamond, Rudolph, Student, Syracuse University.
Doe, Barbara, Instructor in Biology, Loretto Heights College.
Eisenman, George, Student, Harvard University.
Handler, Hope S., Student, Smith College.
Horwitz, Leonard, Student, College of the City of New York.
Lay, Ko Ko, Student, Washington University.
Prodell, Rita C., Student, Drew University.
Rumely, John H., Student, Oberlin College.
Springer, Helen V., Student, Vassar College.
Umanzio, Dr. Carl B., Professor of Bacteria and Public Health, Kirksville College of Osteopathy.
Williams, Louis G., Associate Professor of Biology, Furman University.

EMBRYOLOGY

Anagnostis, Irene P., Student, New York University.
Clark, Eugenie, Research Assistant, American Museum of Natural History.
Clark, Edward C., Student, University of Massachusetts.
Danes, Betty, Student, Mount Holyoke College.
Daniels, Edward W., Research Assistant, University of Illinois.
Easterling, George R., Assistant Professor of Biology, Kent State University.
Haffner, Rudolph E., Student, Yale University.
Healy, Eugene A., Student, Columbia University.
Heath, H. Duane, Student, University of Chicago.
Hodgson, Edward S., Jr., Junior Instructor, Johns Hopkins University.
Jaffee, Oscar C., Student, New York University.
Jaskoski, Benedict J., Student, University of Minnesota.
Jones, Edward E., Student, University of North Carolina.
Kent, John F., Assistant in Zoology, Cornell University.
Moulton, James M., Graduate Assistant in Biology, Williams College.
Nace, George W., Graduate Student, University of California, Los Angeles.
Nadeau, Rev. Louis V., Biology Department, Fenwick High School.
Opperman, Jean Ann, Student, Seton Hill College.
Parks, Harold F., Teaching Assistant, Cornell University.
Raecke, Marjorie J., Graduate Student, University of Nebraska.
Rauch, Harold, Graduate Assistant, Brown University.
Reich, Edward, Undergraduate Assistant, McGill University.
Rhodes, Stanley A., Graduate Assistant, Duke University.
Rossetti, Fiammeta, Student, University of Chicago.
Rothberg, Harvey D., Jr., Undergraduate, Princeton University.
Schreiman, Evelyn S., Assistant Instructor, Rutgers University.
Todd, Doris J., Student, Smith College.
Washington, Dorothy A., Student, George Washington University.
Watson, Ruby J., Student, Wheaton College.

PHYSIOLOGY

Bauer, M. H., Graduate Student, Princeton University.
Bernstein, Maurice H., Graduate Assistant, Washington University.
Boné, George J., Lecturer, Institute of Tropical Medicine, Antwerp, Belgium.
Brigham, Elizabeth H., Student, Rockefeller Institute for Medical Research.
Cheng, Sze-chuh, Graduate Student, Brown University.
Diermeier, Harold F., Graduate Assistant, Syracuse University.
Flagler, Elizabeth A., Student, Mount Holyoke College.
Green, Frances, Teaching Fellow, New York University.
Heroux, Olivier, Graduate Student, Laval University, Quebec.
Hoffman, Joseph F., Student, University of Oklahoma.

Jacobson, Julius H., II, Graduate Student, University of Pennsylvania.
Jencks, William P., Student, Harvard Medical School.
Johnson, Audrey C., Graduate Student, Brooklyn College.
Junqueira, Luiz C. U., Associate Professor, University of São Paulo, Brazil.
Kirschner, Leonard B., Research Assistant in Physiology, University of Wisconsin.
Kuff, Dr. Edward L., Instructor, Washington University Medical School.
Lessler, Milton A., Teaching Fellow, New York University.
Metz, Bernard, Graduate Assistant, Fordham University.
Palay, Sanford L., Visiting Investigator, Rockefeller Institute for Medical Research.
Pardee, Arthur Beck, Post-Doctoral Fellow, University of Wisconsin.
Pease, Evelyn A., Teaching Fellow, University of Michigan.
Rankin, Eugene M., Graduate Student, Tufts College.
Sutro, Peter J., Graduate Student, Harvard University.
Tarr, Elizabeth H., Student, Stanford University.
Zeidman, Irving, Instructor, University of Pennsylvania School of Medicine.

ZOOLOGY

Aldrich, Frederick A., Undergraduate Student Assistant, Drew University.
Armstrong, Ruth A., Student, Vassar College.
Bair, Thomas D., Assistant, University of Illinois.
Barber, Donald S., Graduate Student, Amherst College.
Barnett, Robert Charles, Student, University of Chicago.
Barrett, James M., Graduate Assistant in Zoology, Marquette University.
Bellinger, Peter F., Student, Yale University.
Berger, Ruth, Undergraduate Student, Goucher College.
Bohrn, Marie T., Instructor in Geology, Mount Holyoke College.
Boucot, Arthur J., Student, Harvard University.
Brown, Harley P., Assistant Professor, University of Oklahoma.
Burch, Charles, Graduate Assistant, Cornell University.
Coady, Martha B., Student, Simmons College.
Corliss, John O., Graduate Assistant, New York University.
Deardorff, Alice A., Graduate Assistant, Wesleyan University.
Eckl, Betty A., Student, Mount Holyoke College.
Etz, Monica Zelda, Undergraduate Student, Goucher College.
Ewing, Mary J., Student, Pennsylvania College for Women.
Farrow, Audrey P., Student, Wheaton College.
Ferguson, Anne V. D., Student, Elmira College.
Godshalk, Elizabeth L., Student, University of California, Los Angeles.
Guyselman, John B., Graduate Assistant, Northwestern University.
Hensley, Ruth A., Graduate Assistant in Zoology, University of Missouri.
Holland, Robert A., Student, Drury College.
Holz, George G., Jr., Graduate Assistant in Biology, New York University.
Huntington, Charles E., Student, Yale University.
Ichikawa, Hiroko, Student, Wilson College.
Jenner, Charles E., Graduate Student, Harvard University.
Keevil, Charles S., Jr., Graduate Assistant, Amherst College.
Ketterer, John J., Graduate Assistant in Biology, New York University.
Kuta, Virginia A., Student, DePaul University.
Lambert, Francis L., Student Assistant in Zoology, George Washington University.
Lewis, Franklin B., Student, Union College.
Loud, Alden V., Student, Massachusetts Institute of Technology.
McKibben, Juliet N., Instructor, Carnegie Institute of Technology.
Mirsky, Reba, Student, Indiana University.
Nerad, Josephine, Graduate Student, DePaul University.
Nese, Rose M., 409 Western Avenue, East Pittsburgh, Pennsylvania.
O'Malley, Benedict B., Instructor in Botany and Anatomy, Fordham University.
Orski, Barbara, Student, Hunter College.
Peloquin, Stanley J., Student, Marquette University.

Perry, Thomas O., Student, Harvard University.
Provasoli, Luigi, Research Associate, Haskins Laboratories.
Reichart, Ruth, Student, Radcliffe College.
Rogers, Kay T., Teaching Fellow, Harvard University.
Sachs, Barbara C., Student, Oberlin College.
Shomay, David, Student, Long Island University.
Slobodkin, Lawrence B., Graduate Student, Yale University.
Stevens, Arthur L., Research Associate, University of Notre Dame.
Thomas, Lyell J., Jr., Student, Oberlin College.
Urey, Gertrude E., Student, Swarthmore College.
Willcox, Barbara L., Student, Oberlin College.
Wilson, Carolyn E., Graduate Assistant, State University of Iowa.
Wunder, Charles C., Student, Washington and Jefferson College.

MARINE BIOLOGICAL LABORATORY LIBRARY WOODS HOLE, MASS.

3. The Lalor Fellows

Senior Fellow: Ernest Baldwin, University of Cambridge, England.
Junior Fellows: Avram Goldstein, Harvard Medical School.
Joseph Lein, Syracuse University.
Harold Persky, Michael Reese Hospital, Chicago.
Warren Yudkin, Yale Medical School.
Reappointments: Irving Klotz, Northwestern University.
Arnold Lazarow, Western Reserve.
Benjamin Libet, University of Chicago.
Claude Villee, Harvard Medical School.

4. The Atomic Energy Commission Fellows

Richard Abrams, Institute of Radiobiology, University of Chicago.
D. E. Copeland, Brown University.
T. P. Ting, University of Chicago.
J. J. Wolken, University of Pittsburgh.
A. A. Woodward, Brown University.

5. Tabular View of Attendance

	1944	*1945*	*1946*	*1947*	*1948*
Investigators—Total	193	212	267	299	326
Independent	112	138	175	182	183
Under instruction	11	10	29	36	42
Library readers	50	38	38	36	50
Research assistants	20	26	25	45	51
Students—Total	75	96	122	131	123
Zoology	37	55	57	55	54
Embryology	23	23	30	33	29
Physiology	10	13	26	26	25
Botany	5	5	9	17	15
Total Attendance	276	308	389	430	449
Less persons registered as both students and investigators	1			2	6
	275				443
Institutions Represented—Total	106	124	141	148	158
By investigators	74	100	102	114	117
By students	41	49	56	56	68
Schools and Academies Represented					
By investigators	1	2	2	1	3
By students	2	2		1	
Foreign Institutions Represented					
By investigators	2	1	7	7	8
By students	3		5	3	4

6. Subscribing and Cooperating Institutions, 1948

Cooperating Institutions

Amherst College
Boston Dispensary
Brooklyn College
Brown University
Bryn Mawr College
Carnegie Inst. of Washington
Catholic University of America
College of Mt. St. Joseph-on-the-Ohio
College of Physicians and Surgeons
Columbia University
Cornell University
Duke University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins University
Johns Hopkins University Medical School
Johnson Foundation
Eli Lilly Company
Madison Foundation
Massachusetts Inst. of Technology
Miami University
Mount Holyoke College
Mount Sinai Hospital
New York University College of Medicine
New York University School of Dentistry
New York University Washington Square College
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Rockefeller Foundation
Rockefeller Institute for Medical Research
Seton Hill College
Sloan-Kettering Institute
Smith College
State University of Iowa
Syracuse University Medical School
Temple University
Tufts College Medical School
Union College
University of Chicago
University of Illinois
University of Kansas
University of Maryland School of Medicine
University of Michigan
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Rochester
University of Vermont Medical School
University of Virginia
Vassar College
Washington University
Washington Univ. School of Dentistry
Wesleyan University
Western Reserve University
Woods Hole Oceanographic Institution
Yale University

Subscribing Institutions

Argonne National Laboratory
Belgium American Educational Fund
California Institute of Technology
College of Charleston
College of the City of New York
DesMoines Still College of Osteopathy
Drury College
Elmira College
Georgetown University School of Medicine
Hunter College
Institute for Cancer Research
Institute for Muscle Research
Institute of Pennsylvania Hospital
W. K. Kellogg Foundation
Marquette University
National Cancer Institute
National Institute of Health
North Carolina State College of Agriculture & Engineering
Public Health Research Institute of New York City
Radcliffe College
Southwestern Medical College
University of Delaware
University of Massachusetts
University of Oklahoma
University of Pittsburgh
Wheaton College

7. The Friday Evening Lectures, 1948

Friday, July 2
Dr. William R. Duryee .... "The Structure and Function of Egg Nuclei."
Friday, July 9
Dr. J. T. Bonner .... "Morphogenetic Movements in the Amoeboid Slime Molds."

Friday, July 16	
Dr. Ernest Baldwin	"Nemathelminthes and Antihelminthics."
Friday, July 23	
Dr. Paul C. Aebersold	"Recent Developments in the Use of Isotopes in Biology and Medicine."
Friday, July 30	
Dr. A. H. Sturtevant	"Closely Similar Genes at Identical Loci."
Friday, August 6	
Dr. John O. Hutchens	"Biological and Chemical Activities of Nitrogen Mustards."
Friday, August 13	
Prof. E. W. Sinnott	"The Problem of Size Determination in Plants."
Friday, August 20	
Dr. Paul Weiss	"Nerve Growth and the Problem of Synthesis of Protoplasm."
Friday, August 27	
Prof. Karl F. Bonhoeffer	"The Mechanism of Chemical Rhythmical Reaction."

Other Lectures

Wednesday, August 4	
Dr. L. V. Foster	"Advance in Optical Instrumentation."
Thursday, August 12	
Dr. George Lower	"Kodachromes of Marine Life at Woods Hole and Bermuda."
Wednesday, August 18	
Dr. P. S. Galtsoff	"Biological Explorations in the Gulf of Panama."

8. Seminars, 1948

July 6	
Arnold Lazarow	"Sulfhydral Metabolism of the Beta Cell: Its Relation to the Development of Diabetes."
Shlomo Hestrin	"Action Pattern of Crystalline Muscle Phosphorylase."
Rudolph Keller	"Vital Staining in Combined Ultra Violet and Daylight."
July 13	
Milton Levy and Evelyn Slobodiansky	"The Arrangement of Amino Acids in Silk —an Application of Isotopic Derivative Analysis."
Anita Zorzoli	"The Histochemical Localization of Alkaline Phosphatase in Mouse Bones of Different Ages."
B. Eichel, S. Cooperstein and W. W. Wainio	"A Partial Separation of the Cytochromes."
July 20	
K. B. Augustinsson	"On the Specificity of Cholinesterase."
David Nachmansohn	"Effect of Anticholinesterases in conduction."
M. A. Rothenberg	"Rate of Electrolyte Penetration into the Nerve Interior."

July 27

F. W. Whiting and Bertina M. Blauch	"Reproductive Economy and the Genetic Block to Free Oviposition in the Chalcidoid Wasp, Melittobia."
Luiz C. V. Junqueira	"Biochemical and Histochemical Observation on the Sexual Dimorphism of Mice Submaxillary Glands."
Alfred E. Bliss	"Extraction of Purified Squid 'Visual Purple.' "
James A. Miller	"PH Estimations in Reconstituting Tubularia Stems."

August 3

Ivor. Cornman	"Inhibition of Sea Urchin Egg Cleavage by a Series of Substituted Carbamates."
Alfred Marshak	"A Nuclear Precursor to Ribo- and Desoxyribonucleic Acids."
A. M. Shanes	"The Effects of Excitatory and Blocking Mechanism Supporting the Resting Potential of Nerve."

August 10

James W. Green	"The Relative Rate of Penetration of the Lower Fatty Acids into Beef Red Cells."
F. R. Hunter	"Osmotic Hemolysis in Hypertonic Solutions."
Harold Persky	"Hippuric Acid Excretion in Anxiety States."
Dorothy Wrinch	"Biological Specificity."

August 17

R. K. Crane, E. G. Ball, and A. K. Solomon	"Incorporation of Carbon Dioxide into Organic Linkage by Retina."
Claude A. Villee, M. Lowens, M. Gordon, E. Leonard, and A. Rich	"The Synthesis of Nucleoproteins in Developing Arbacia Studied with the Aid of P32."
Avram Goldstein	"Mechanisms of Interaction of Inhibitors with Plasma Cholinesterase."
E. DeRobertis	"Ultrastructure of the Nerve Axon."

9. MEMBERS OF THE CORPORATION, 1948

1. LIFE MEMBERS

Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.

Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mrs. T. H., Pasadena, California.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Scott, Dr. Ernest L., Columbia University, New York City, New York.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Strong, Dr. O. S., Columbia University, New York City, New York.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.
Albaum, Dr. Harry G., Biology Dept., Brooklyn College, Brooklyn, N. Y.
Albert, Dr. Alexander, Mayo Clinic, Rochester, Minnesota.
Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.
Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine, Baltimore, Md.
Anderson, Dr. Rubert S., Department of Physiology, University of South Dakota, Vermillion, South Dakota.
Anderson, Dr. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.
Angerer, Prof. C. A., Department of Physiology, Ohio State College, Columbus, Ohio.
Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.
Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts.
Baitsell, Dr. George A., Yale University, New Haven, Connecticut.
Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.
Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire.
Ballentine, Dr. Robert, Columbia University, Department of Zoology, New York City, New York.
Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Maryland.
Barron, Dr. E. S. Guzman, Department of Medicine, The University of Chicago, Chicago, Illinois.
Barth, Dr. L. G., Department of Zoology, Columbia University, New York City, New York.
Bartlett, Dr. James H., Department of Physics, University of Illinois, Urbana, Illinois.

Beadle, Dr. G. W., California Institute of Technology, Pasadena, California.

Beams, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Beck, Dr. L. V., Edgey Road and Beech Avenue, Bethesda, Maryland.

Beers, C. D., University of North Carolina, Chapel Hill, North Carolina.

Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, Louisiana.

Bertholf, Dr. Lloyd M., College of the Pacific, Stockton, California.

Bevelander, Dr. Gerrit, New York University School of Medicine, New York City, New York.

Bigelow, Dr. H. B., Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

Bissonnette, Dr. T. Hume, Trinity College, Hartford, Connecticut.

Blanchard, Prof. K. C., Johns Hopkins Medical School, Baltimore, Maryland.

Bliss, Dr. Alfred F., Department of Physiology, Tufts College Medical School, Boston, Mass.

Blum, Dr. Harold F., Department of Biology, Princeton University, Princeton, New Jersey.

Bodine, Dr. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Boell, Dr. Edgar J., Yale University, New Haven, Connecticut.

Bonner, Dr. John T., Department of Biology, Princeton University, Princeton, New Jersey.

Boring, Dr. Alice M., Yenching University, Peiping, China.

Bradley, Prof. Harold C., University of Wisconsin, Madison, Wisconsin.

Brodie, Mr. Donald M., 522 Fifth Avenue, New York City, New York.

Bronfenbrenner, Dr. Jacques J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.

Bronk, Dr. Detlev W., Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania.

Brooks, Dr. Matilda M., University of California, Department of Zoology, Berkeley, California.

Brown, Dr. Dugald E. S., Bermuda Biological Station, St. George's West, Bermuda.

Brown, Dr. Frank A., Jr., Department of Zoology, Northwestern University, Evanston, Illinois.

Brownell, Dr. Katherine A., Ohio State University, Columbus, Ohio.

Buck, Dr. John B., Industrial Hygiene Research Lab., National Institute of Health, Bethesda, Maryland.

Buckingham, Miss Edith N., Sudbury, Massachusetts.

Budington, Prof. R. A., Winter Park, Florida.

Bullington, Dr. W. E., Randolph-Macon College, Ashland, Virginia.

Bullock, Dr. T. H., University of California, Los Angeles 24, California.

Burbanck, Dr. William D., Department of Biology, Drury College, Springfield, Missouri.

Burkenroad, Dr. M. D., Central Park W. at 79th Street, New York City, New York.

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STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. IV. EFFECT OF X-RAY IRRADIATION ON THE RESPIRATION OF SEA URCHIN SPERM

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It has been maintained by a number of investigators (see reviews by Scott, 1937; Fricke, 1934; Packard, 1931) that the respiration of single cells is strikingly resistant to the action of ionizing radiations, and this belief has been the basis for ignoring the role of enzyme inhibition when explaining the mechanism of action of ionizing radiations on living cells. In fact, Chesley (1934) reported that the respiration of sea urchin eggs, both fertilized and unfertilized, was unaffected by x-ray irradiation with as much as 43,000 r. Henshaw (1932, 1940) found delay in cleavage whether the eggs or the sperm were irradiated, but the smallest amount of irradiation used in these experiments was 4000 r. Evans et al. (1942) confirmed Henshaw's work on sea urchin sperm and reported that inhibition of fertilization by x-rays could be partially prevented by the addition of certain organic substances, as had previously been found by Dale (1940) when he discovered this protective effect against enzyme inhibition by x-rays. In previous reports it has been shown that the respiration of tissue slices from rats irradiated with doses of x-rays below 500 r was definitely inhibited (Barron, 1947) and that the respiration of grasshopper eggs was inhibited with doses of x-rays as low as 10 r (Tahmisian and Barron, 1946). It was considered important, in view of these last experiments, to reinvestigate the problem of the respiration of irradiated single cells. We present in this paper data on the effect of x-rays on the respiration of sea urchin (*Arbacia punctulata*) sperm.

EXPERIMENTAL

Sperm was obtained by cutting circularly the soft tissues of the sea urchin. By this process sperm was shed in small syracuse dishes. Sperm from several urchins was collected in graduated centrifuge tubes after filtration through gauze. Filtered sea water was added to fill up the centrifuge tube. Sperm was then separated by short centrifugation (10 minutes at 2000 r.p.m.), the supernatant fluid was discarded, and the remaining sperm was brought to the desired dilution starting with a stock dilution of 1:10 or 1:20. The stock suspension was thoroughly shaken and an aliquot was taken for dry weight. To obtain the dry weights, 0.5 cc. of this suspension was added to specially hardened pyrex tubes and was centrifuged in a Beams type air-driven high speed centrifuge with 85 lbs. air pressure for 10 minutes. The fluid was withdrawn and the tubes were dried overnight at 110°. The sperm dilution chosen for the irradiation experiments was 1:200. This sperm suspension (1.2 cc.) was pipetted into small glass vials of 15 mm. diameter and 20 mm. height.

These vials (20 for each set of experiments) were placed in an aluminum holder resting in a glass container full of cracked ice. X-ray irradiation was performed by Mr. Hyde of the Department of Radiology of the Marine Biological Laboratory. The x-ray machine operated at 182 Kv peak voltage and a current of 25 ma. through each tube. A filter of 0.2 mm. Cu was used. The measurement of respiration started 40 minutes after irradiation.

Effect of dilution on the respiration of sea urchin sperm

Gray found in 1928 that the respiration of sea urchin sperm increased on dilution, but no quantitative study of this phenomenon has yet been reported. Although Hayashi (1946) attempted to measure this dilution effect, his techniques of measurement of O_2 uptake and of dilution were faulty, and his paper gives no data but rough figures. It was, therefore, necessary to determine the optimum sperm dilution which would give the maximum Q_{O_2} values (c.mm. of O_2 uptake per mg. dry weight per hour), and steady rates of respiration for at least one hour. A large number of experiments were performed for this purpose with different sperm dilutions, from 1:10 to 1:1000. The O_2 uptake increased steadily up to a dilution of 1:200. It started to decline when the dilution was increased to 1:400. A dilution of 1:1000 gave an O_2 uptake as small as that of sperm at a dilution of 1:10; furthermore, the respiration almost ceased at the end of one hour. When the sperm was diluted to 1:1600, there was no measurable respiration (Table I). This lack of respiration was not due to lack of sensitivity of the Warburg manometric technique, for when measurements were made with the very sensitive Cartesian diver technique of Linderstrom-Lang as modified by Claff [1] similar negative results were obtained. The increase in respiration of sperm with dilution is undoubtedly due to greater motility in the dilute solutions.

TABLE I

The effect of dilution on the respiration of sea urchin sperm (Arbacia punctulata). *Values, Q_{O_2}, give c.mm. O_2 uptake per mg. dry weight per hour*

Dilution	Q_{O_2}
1:10	1.7
1:30	3.6
1:100	10.0
1:150	14.6
1:200	19.6
1:400	17.5
1:1,000	2.0
1:1,600	None

When experiments were performed on one sample of pooled sperm, the values agreed within 10 per cent. The experiments performed on successive days with different sperm suspensions and the same dilution did not give reproducible values. The average Q_{O_2} value of 29 separate experiments performed on separate days (each experiment in triplicate) with a sperm dilution of 1:200 was 19.6 ± 3.9, i.e., with 20 per cent variation. Sperm dilutions of 1:200 could be kept at 3° for six hours with little decrease in respiration (11 to 14 per cent).

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[1] Personal communication.

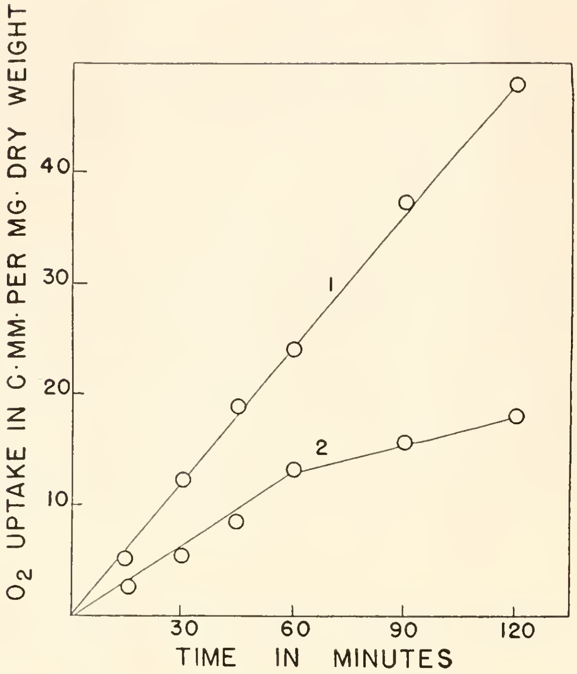

FIGURE 1. The effect of x-ray irradiation on the respiration of sea urchin sperm. Irradiation, 20,000 r. 1. Control; 2. Irradiated sperm.

It was not possible to obtain similar values in the O_2 uptake when the sperm was suspended in boiled sea water or in artificial sea water. The respiration of sperm in these cases was always much lower (20 to 40 per cent less).

Effect of x-rays on the respiration of sea urchin sperm

Once it was established that the optimum dilution for the measurement of sperm respiration was 1:200, all the experiments on the effect of x-ray irradiation were

TABLE II

Effect of x-rays on the respiration of sea urchin sperm. Dilution 1:200. Irradiation, 20,000 r. Figures give c.mm. O_2 uptake per mg. dry weight per hour

Exp. no.	Control	Irradiated	Inhibition (per cent)
1	14.8	6.0	59.4
2	16.2	3.8	76.6
3	12.0	5.0	58.4
4	23.9	8.9	62.8
5	18.3	5.3	71
6	13.1	2.8	79
7	29.6	17.6	54
8	18.6	10.6	43

performed with sperm suspensions so diluted. The sperm suspensions from 20 irradiated vials were collected in an Erlenmeyer flask from which 3 cc. were pipetted to each Warburg vessel. Thus, every experiment consisted of six control vessels and six vessels with irradiated sperm. Because of the reports in the literature on the resistance of respiration to x-ray irradiation, it was decided to start with 20,000 r. Such irradiation produced a marked inhibition of respiration, which increased in the second hour (Fig. 1). The degree of inhibition varied from sample to sample in all experiments. As an example of this variation the data of experiments of irradiation with 20,000 r are given in Table II. From this x-ray dose the amount of irradiation was diminished to 100 r with which an inhibition of 10 per cent was observed (Table III). The O_2 uptake inhibition on irradiation with 100 r did not increase in the second hour after irradiation (Fig. 2). In previous work

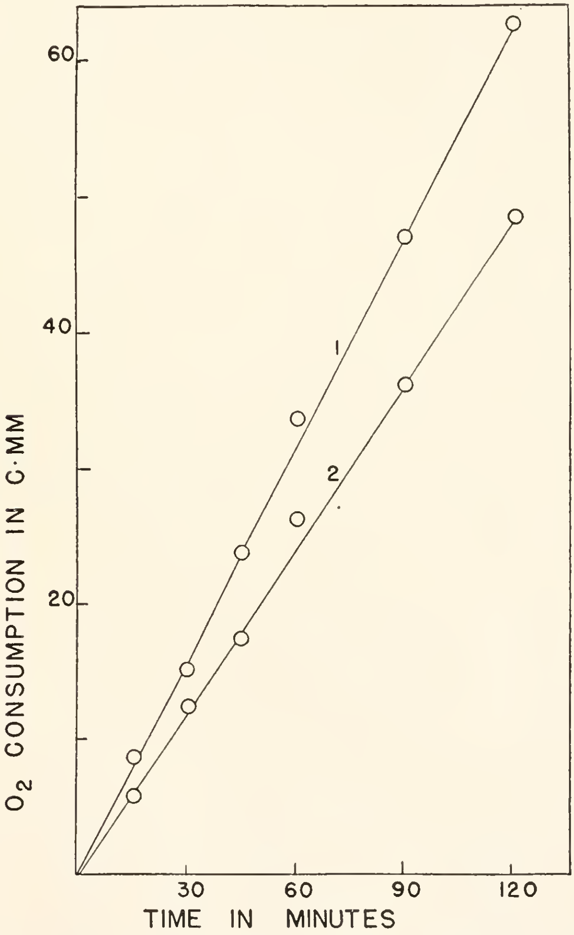

FIGURE 2. The effect of x-ray irradiation on the respiration of sea urchin sperm. Irradiation, 100 r. 1. Control; 2. Irradiated sperm.

TABLE III

The effect of x-rays on the respiration of sea urchin sperm. Sperm dilution 1:200. Q_{O_2} values, c.mm. O_2 uptake per mg. dry weight per hour

X-ray dose (r)	Q_{O_2} values		Inhibition (per cent)
	Control (c.mm.)	X-ray (c.mm.)	
20,000	22	7.5	66
10,000	19	13.3	30
1000	28	19.8	22
500	18	15.5	14
100	20	18	10

with grasshopper eggs irradiated with small doses of x-rays, there was inhibition of respiration when measurement of the O_2 uptake was made soon after irradiation, but a return to normal values five hours after irradiation (Tahmisian and Barron, 1947). Several attempts were made to see whether the respiration of sea urchin sperm inhibited by x-rays could also recover a few hours after irradiation. Soon after irradiation the sperm-containing vials were kept at 3° for five hours and the respiration was then measured. The degree of inhibition was the same as that obtained when measurements were made soon after irradiation.

The inhibition of respiration produced by x-ray doses between 1000 r and 100 r could not be attributed to H_2O_2 formation (even if there were H_2O_2 formation on irradiation of sea water) because small amounts of H_2O_2 increased respiration. Furthermore, a portion of this H_2O_2 would be destroyed by sperm catalase (Fig. 3). The catalase content of sea urchin sperm was such that 1 mg. dry weight would produce 33 c.mm. O_2 per hour at 25°, a figure which is eleven times less than the catalase content of mouse testicle. For these experiments sperm was washed three times in sea water, it was suspended in 10 volumes of water, and homogenized. Sperm thus treated gave no O_2 uptake.

TABLE IV

Effect of x-rays on the oxidation of succinate and acetate by sea urchin sperm. Substrate concentration, 0.01 M

X-ray dose (r)	Substrate	Q_{O_2} values		Inhibition (per cent)
		Control (c.mm.)	Irradiation (c.mm.)	
100	None	22.2	19.3	13
100	Succinate	30.0	22.8	24
100	None	20.5	18.0	12
100	Acetate	25.0	18.3	27
1000	None	20.5	15.0	26.9
1000	Succinate	27.2	20.0	38

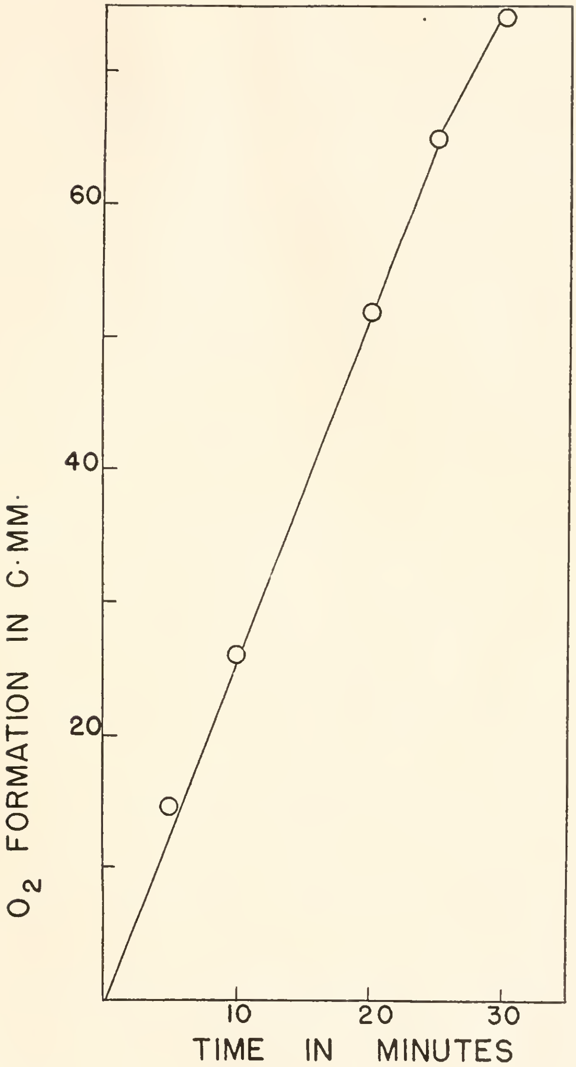

FIGURE 3. Catalase in sea urchin sperm. Buffer, phosphate, 0.01 M, pH 6.8; H_2O_2 0.01 M. Temperature 25°; Sperm, 0.1 cc. of 1 : 10 suspension in H_2O, 1.8 mg. dry weight.

A number of intermediary metabolites are utilized by sea urchin sperm with an increase in the O_2 uptake. Of these, the utilization of succinate and of acetate (as measured by the increase of O_2 uptake) was tested after irradiation with 100 r. In both cases the inhibition of O_2 uptake was greater in the presence of substrates (Table IV), an indication that the enzymes for the oxidation of succinate and of acetate which belong to the group of sulfhydryl enzymes, are quite sensitive to the inhibiting effect of x-rays.

SUMMARY

The respiration of sea urchin (*Arbacia punctulata*) sperm increased with dilution up to a dilution of 1:200, where maximum values were found. At this dilution the average Q_{O_2} value was 19.6 ± 3.9. When the dilution was increased to 1:1000 the respiration dropped sharply to 2.0. A dilution of 1:1600 gave no measurable respiration.

The respiration of dilute suspensions of sea urchin sperm (1:200) was inhibited by x-ray irradiation. A dose of 20,000 r produced an inhibition of 66 per cent which was further increased during the second hour; 10,000 r inhibited 30 per cent; 1000 r, 22 per cent; 500 r, 14 per cent; and 100 r, 10 per cent. When sperm was irradiated with 1000 r there was no recovery of respiration five hours after irradiation. Inhibition of respiration cannot be attributed to hypothetical H_2O_2 formation, for sperm suspensions contain catalase. The catalase value of sperm is 33 c.mm. O_2 formed by 1 mg. dry weight per hour, i.e., 3 micromoles H_2O_2 destroyed. On addition of succinate and of acetate to sperm irradiated by x-rays the O_2 uptake inhibition increased.

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STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. V. THE EFFECT OF HYDROGEN PEROXIDE AND OF X–RAY IRRADIATED SEA WATER ON THE RESPIRATION OF SEA URCHIN SPERM AND EGGS

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It has been known for some time (Risse, 1929; Fricke, 1934) that when water is irradiated with x-rays in the presence of oxygen there is formation of H_2O_2. Since H_2O_2 is a powerful oxidizing agent and it easily oxidizes sulfhydryl groups, it was reasonable to assume that this substance, if formed on irradiation, would contribute to the biological effects of ionizing radiations. In fact, Barron and Dickman (1949) on studying enzyme inhibitions by ionizing radiations, and Barron and Flood[1] on studying the oxidation of 2,3-dithiopropane and of glutathione by x-rays, were able to distinguish the H_2O_2 contribution to this oxidation by the use of catalase. The French investigators Loiseleur, Latarjet, and Caillot (1941), and Loiseleur and Latarjet (1942) have postulated that the primary effect of irradiation in aqueous solutions is H_2O_2 formation, which would thus become of importance in the interpretation of the mechanism of ionizing radiations. The same view is held by Evans (1947) who found that the fertilizing power of sea urchin sperm is decreased when suspended in sea water irradiated with large doses of x-rays. Evans attributed this inhibition to the action of H_2O_2 seemingly formed on irradiation of sea water. In living cells the role of H_2O_2 becomes more complicated because the sulfhydryl groups which might be oxidized by this agent not only are present in the protein moiety of enzymes, but also exist as non-protein sulfhydryl groups.

We present in this paper experiments on the effect of H_2O_2 and of x-ray irradiated sea water on the respiration of sea urchin sperm. They do not support the belief that H_2O_2 is an important factor in x-ray toxicity on sea urchin sperm.

EXPERIMENTAL

Sea urchin sperm was obtained as described previously (Barron et al., 1949), and in all experiments a dilution of 1 : 200 was used. Freshly filtered sea water was irradiated at room temperature in large cellophane dishes and immediately after irradiation the sperm suspension was added, enough to make the desired dilution of 1 : 200.

The catalase added to irradiated sea water was prepared from beef liver according to Sumner and Dounce (1939). H_2O_2 was determined by the colorimetric method of Bonet-Maury (1944). An aliquot of the solution was taken (up to

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[1] Unpublished experiments.

3 cc.) to which was added 0.5 cc. of 20 per cent H_2SO_4, 5 drops of the titanium sulfate reagent (10 g. $TiSO_4$ ground in mortar with 50 cc. H_2O and 20 g. H_2SO_4, D = 1.84, let stand 24 h., centrifuge, take the supernatant and add 20 g. H_2SO_4), and distilled water to 5 cc. The yellow color produced which is stable was read in a Beckman spectrophotometer at 4000 Å. With this method amounts of H_2O_2 from 0.5 micrograms to 25 micrograms could be determined. The respiration measurements were made at 25°.

Effect of H_2O_2 on the respiration of sea urchin sperm

Barron et al. (1948) have shown that sulfhydryl reagents when used in small concentrations increase the respiration of sea urchin sperm, whereas they inhibit it when the concentration is increased. To explain these opposite effects it was postulated that the cell contains two kinds of sulfhydryl groups: the non-protein sulfhydryl groups (namely glutathione), which regulate the rate of respiration, and the sulfhydryl groups in respiratory enzymes. Destruction of the first would increase respiration, while destruction of the second would inhibit it. H_2O_2, an oxidizing agent of sulfhydryl groups, behaves in the same manner. At a concentration of 1×10^{-5} M it increased respiration, while 1×10^{-3} M inhibited it almost completely (Fig. 1). By altering the concentration of H_2O_2 between these two limits the effect on respiration is changed accordingly (Table I). In fertilized sea urchin eggs 1×10^{-4} M H_2O_2 increased the O_2 uptake 25 per cent (Fig. 2). If the effects of x-ray irradiation were mainly due to H_2O_2 formation, as postulated by

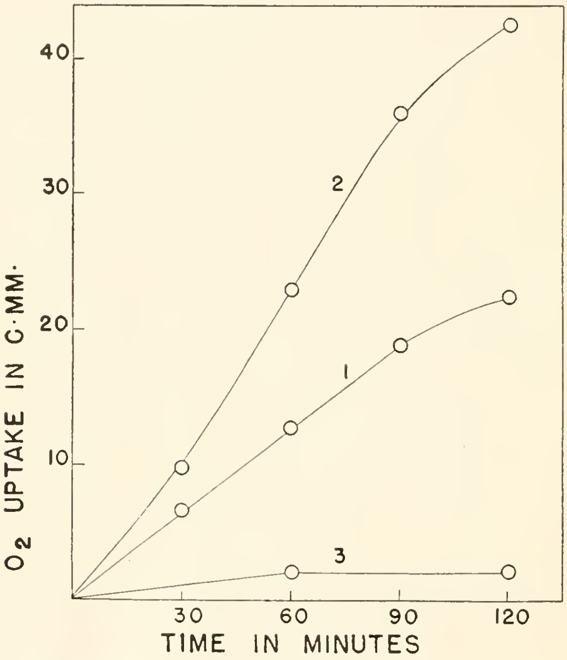

FIGURE 1. The effect of H_2O_2 on the respiration of sea urchin sperm. 1. Control; 2. H_2O_2, 1×10^{-5} M; 3. H_2O_2, 1×10^{-3} M.

TABLE I

Effect of H_2O_2 on the respiration of sea urchin sperm. Sperm dilution, 1:200.
Q_{O_2}, c.mm. O_2 uptake per mg. dry weight per hour

H_2O_2 Concentration (*M*)	Q_{O_2} values		Inhibition (−) or increase (+) (per cent)
	Control (c.mm.)	H_2O_2 (c.mm.)	
0.01	22.3	0	− complete
0.001	22.3	2.1	−90
0.0005	16.0	15.3	No effect
0.0001	20.2	33.7	+61.5
0.0001	22.0	42.0	+91

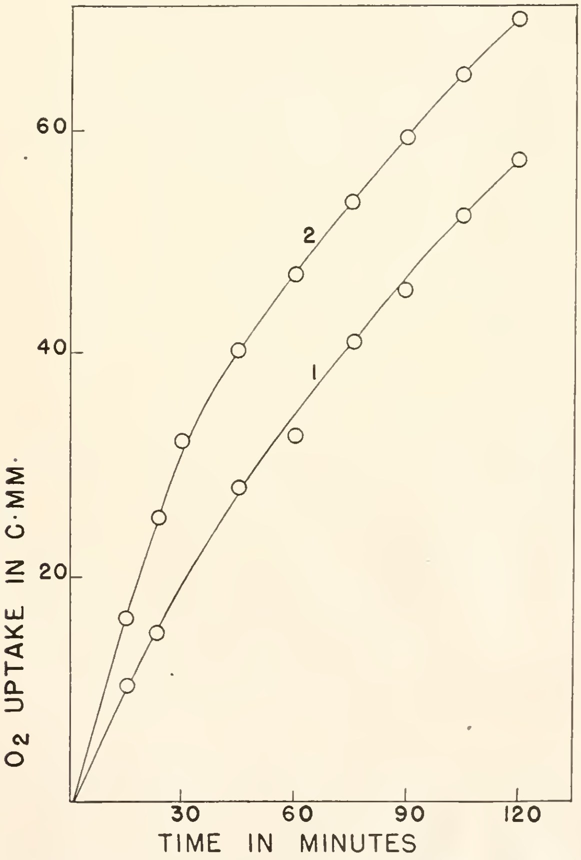

FIGURE 2. Effect of H_2O_2 on the respiration of fertilized sea urchin eggs. H_2O_2 concentration, 1×10^{-4} M; 1. Control; 2. H_2O_2.

TABLE II

Inhibition of sea urchin sperm respiration by x-ray irradiated sea water. X-ray dose, 100,000 r. Liver catalase (0.2 cc.) added immediately after irradiation; sperm suspension 5 minutes later

Experimental conditions	O_2 uptake		Inhibition (per cent)
	First hour (c.mm.)	Second hour (c.mm.)	
Control	29.7	53.5	
X-ray irradiated sea water	14.9	33.1	38
X-ray irradiation + catalase	16.2	36.6	34

Loiseleur et al. (1941, 1942) and by Evans (1947), x-ray irradiation at a dose of 1000 r would produce an increase in cell respiration.[2] Barron et al. (1949) have shown that on the contrary, respiration is inhibited. It must be concluded from these experiments that the effects of x-ray irradiation on the metabolism of sea urchin sperm cannot be attributed to H_2O_2 formation.

Effect of x-ray irradiated sea water on the respiration of sea urchin sperm

A number of investigators have reported that on irradiation of aqueous solutions, whether with x-rays or with ultra-violet light, there is formation of some unknown substance which will produce inhibition of growth of protozoa (Taylor et al., 1933), inhibition of the fertilizing capacity of sea urchin sperm (Evans, 1947), inhibition of the growth of bacteria (Wyss et al., 1948), and increase in the mutation rate of *S. aureus* to penicillin resistance (Stone et al., 1947). When freshly filtered sea water was irradiated with 100,000 r, and a suspension of sperm was added to it soon

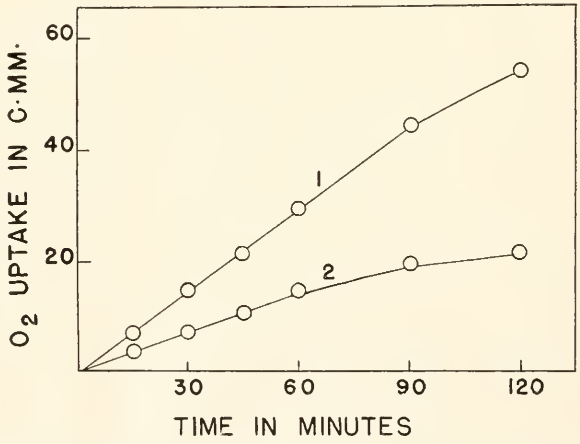

FIGURE 3. Inhibition of sea urchin sperm respiration by x-ray irradiated sea water. Irradiation, 200,000 r. 1. Sperm suspended in sea water; 2. Suspended in x-ray irradiated sea water.

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[2] From Bonet-Maury and Frilley's data (1944) it can be calculated that 1000 r would produce on irradiation of water about 1×10^{-5} M of H_2O_2.

after irradiation, there was an inhibition of respiration of 38 per cent. The inhibition was not affected by previous addition of catalase (Table II), an indication that the inhibition was not produced by H_2O_2. The inhibition increased when the dose of x-rays rose to 200,000 r (Fig. 3). Further evidence that this phenomenon was not produced by H_2O_2 was obtained by its detection with the titanous sulfate colorimetric method. While distilled water irradiated with 100,000 r gave 30 micrograms H_2O_2 per cc., filtered sea water irradiated with the same dose of x-rays gave no color at all. The lack of color formation was not due to the salt concentration of sea water, for on addition of H_2O_2 to sea water the color reaction appeared. We believe that the inhibition of respiration is due to the formation of stable organic peroxides formed on oxidation of the organic matter contained in sea water. It is quite possible that similar stable organic peroxides are formed on irradiation of biological fluids and that they contribute to the toxic effects of ionizing radiations. This problem is now under investigation.

SUMMARY

Hydrogen peroxide at a concentration of 0.001 M produced almost complete inhibition of the respiration of sea urchin sperm suspended in sea water. At a concentration of 0.0005 M it had no effect. When the concentration was diminished to 0.0001 M it increased the respiration from 60 to 100 per cent.

When sperm was added to sea water irradiated with 200,000 r, there was a marked inhibition of respiration (about 60 per cent). Sea water irradiated with 50,000 r produced small inhibition (10 per cent). Addition of catalase previous to the addition of sperm had no effect at all on this inhibition. Furthermore, sea water irradiated with 200,000 r gave no positive test for H_2O_2. It is postulated that inhibition is due to the action of stable organic peroxides produced on irradiation of sea water.

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BACTERIA AND CELLULAR ACTIVITIES. IV. ACTION OF TOXINS ON RESPIRATION AND HEMOLYSIS OF DOGFISH ERYTHROCYTES AND ON RESPIRATION OF MARINE EGGS [1, 2]

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An attempt is being made in this laboratory to determine what effect bacterial toxins have on the functioning of cells. In the two preceding papers of this series (Hunter et al., 1949a, b) the action of seven toxins on the respiration and permeability of chicken erythrocytes was reported. In the present investigation the erythrocytes of the smooth dogfish, *Mustelus canis* and the unfertilized eggs of *Arbacia punctulata* and of *Asterias forbesei* were the cells studied.

Materials and Methods

The toxins were those previously used which were obtained locally and from the Lilly Laboratories and the Lederle Laboratories, to which the authors are indebted. Bacteriologically sterile techniques were used throughout except during the few minutes when hemolysis measurements were made. Tests for sterility were run at the end of each experiment as previously described (Hunter et al., 1949a). In addition, tests were also made for possible contamination by marine bacteria (see Waksman et al., 1933).

Oxygen consumption measurements were made using a Warburg apparatus at a temperature of 25° ± 0.1° C. Hemolysis times were measured using a photronic cell apparatus and a microammeter, since the more sensitive apparatus usually employed was not available. These measurements were made at room temperature which varied from day to day, but a water jacket surrounding the hemolysis chamber tended to minimize fluctuations during a series of readings. The time for hemolysis in 0.95 M ethylene glycol was measured in all cases.

Blood was procured under sterile conditions either by removal from the caudal vein with a hypodermic syringe, or by cutting the tail and allowing the blood to drain into a flask. Heparin was used as an anticoagulant in all experiments.

Immediately following the withdrawal of blood it was centrifuged at about 2000 r.p.m. for 10 minutes. The plasma and leucocytes were removed and the cells were carefully stirred. To 1 cc. of toxin was added 0.3 or 0.5 cc. of erythrocytes in each Warburg vessel which was immediately connected to its manometer and placed in the bath. Five to ten minutes were allowed for temperature equilibration, so that the initial readings were taken within 20 minutes or less of the time the toxins and cells were mixed. Controls were run using 1 cc. of sea water, 1 cc. of broth,

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[1] This work was supported in part by grants from the Division of Grants and Research, U. S. Public Health Service and the Faculty Research Fund of the University of Oklahoma.

[2] An abstract of a portion of this work appeared in the *Biol. Bull.*, **95**: 255, 1948.

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or 1 cc. of formalized toxins in place of the 1 cc. of toxins. In some experiments readings were made continuously, while in others the manometers were opened for about an hour following the first hour's readings. Cell counts and hematrocrit determinations were made as previously described.

Hemolysis measurements were made as follows: Equal volumes of blood and toxins were mixed and allowed to stand at room temperature. Controls were run substituting sea water, formalized toxins, formalized broth and formalized sea water for the toxins. From time to time 0.1 cc. aliquots were removed and added to 10 cc. of 0.95 M ethylene glycol in the hemolysis chamber. The course of hemolysis was followed by reading the microammeter at five-second intervals. The total exposure time to the toxins varied, but the maximum was 48 hours.

Formalized "controls" were used in both the respiratory and hemolysis experiments. These were the same stock suspensions previously described (Hunter et al., 1949a). As was true in the preceding work (Hunter et al., 1949b), these attenuated toxins were more satisfactory as "controls" for the permeability studies than for the respiratory studies. However, these attenuated toxins were more satisfactory as "controls" in the present respiration studies. Apparently the oxygen consumption of dogfish erythrocytes is not inhibited by formalin to such an extent as the oxygen consumption of chicken erythrocytes.

Unfertilized eggs of *Arbacia punctulata* and of *Asterias forbesei* were obtained under bacteriologically sterile conditions, using a technique similar to that described by Tyler et al. (1938). The dry weight of the eggs was obtained by centrifuging 1 cc. samples of the egg suspensions using an air turbine, removing the supernatant fluid and drying to constant weight.

In the majority of experiments in which eggs were used, 1 cc. of the toxin was placed in each Warburg vessel, 2 cc. of an egg suspension were added, the vessels were attached immediately to the manometers and five minutes were allowed for temperature equilibration. The first reading was taken within 20 minutes of the time the first vessel was prepared. Readings were taken at 10 minute intervals over a period of several hours.

RESULTS

Erythrocyte respiration

The results of a typical experiment in which dogfish erythrocytes were exposed to the toxins are summarized in Table I. Respiration was also measured in the presence of formalized toxins (26 days after mixing formalin and toxin). In the case of the three toxins which accelerate respiration (*M. aureus, Cl. tetani* and *C. diphtheriae*), cells in the presence of the corresponding formalized toxins consumed oxygen at essentially the same rate as sea water controls. The other formalized toxins inhibited the rate of respiration but none more markedly than formalized sea water.

Hemolysis times

The effects of the toxins on hemolysis of dogfish erythrocytes in 0.95 M ethylene glycol are shown in Table II. It can be seen that the toxins of *Cl. perfringens* are most effective in altering the surface of the cells, for a half hour exposure is sufficient to bring about a marked increase in the rate of hemolysis. A 9 hour ex-

TABLE I

A typical experiment showing the rates of respiration of dogfish erythrocytes in the presence of various toxins

Toxin	μL O_2 per cc. of cells per hour		Remarks	
	1st hour	3–5 hours	1st hour	3–5 hours
Control	80	70	———	———
M. aureus	140	108	Marked acceleration	Marked acceleration—slightly less than 1st hour
Cl. tetani	160	85	Marked acceleration	Slight acceleration
C. diphtheriae	120	75	Marked acceleration	Little or no effect
Cl. septicum	80	70	No effect	No effect
Cl. perfringens	80	0	No effect	Complete inhibition (Possibly associated with hemolysis of the cells)
B. cereus	65	60	Slight inhibition	Slight inhibition
Str. pyogenes	50	<20	Moderate inhibition	Marked inhibition

posure to the toxins of *Str. pyogenes* or *B. cereus* or a 23 hour exposure to the toxins of *Cl. tetani* produce a less marked increase in the rate of hemolysis. The formalized tetanal toxins had the same effect as the toxins themselves, while the formalized streptococcal toxins, and to a lesser extent, the formalized cereus toxins, had an intermediate effect. These data might indicate either that the formalin had not completely inactivated the toxins responsible for the change in the surface of the cells, or that the changes in the cells are brought about by something other than the toxins. Although additional experiments would be required to demonstrate conclusively the explanation for these observations, there is little reason for believing that substances other than the toxins would be influencing the cells in this manner. The composition of the broth in this case is unknown. However, none of the broths studied in this laboratory has had any observable influence on the osmotic behavior

TABLE II

Hemolysis times for dogfish erythrocytes in 0.95 M ethylene glycol following exposure to various toxins

Time of exposure in hours	Organism producing toxin	Time in seconds for approximately 75 per cent hemolysis			
		Toxin	Control		
			Sea water	Formalized sea water	Formalized toxin
½	*Cl. perfringens*	11	31	31	29
9	*Str. pyogenes*	20	35	35	24
9	*B. cereus*	21	35	37	29
23	*Cl. tetani*	24	36	38	25
20	*Cl. septicum*	40	40	—	40
20	*C. diphtheriae*	30	35	25	30
20	*M. aureus*	35	35	25	30

of dogfish or other erythrocytes. In the case of tetanal toxin, the data obtained from the oxygen consumption studies suggest that the formalin does inactivate the toxin responsible for the change in respiration. As a tentative suggestion, therefore, one might assume that at least two tetanal toxins are present—one which accelerates respiration and is inactivated by formalin, and a second which alters the surface of the cell but which is not inactivated by formalin. It is hoped that future investigations will test the validity of this assumption.

The toxins of *Cl. septicum, C. diphtheriae,* and *M. aureus* have no effect on hemolysis following exposures of 20 hours.

Egg respiration

Figures 1 and 2 present the results of typical experiments using Arbacia and Asterias eggs respectively.

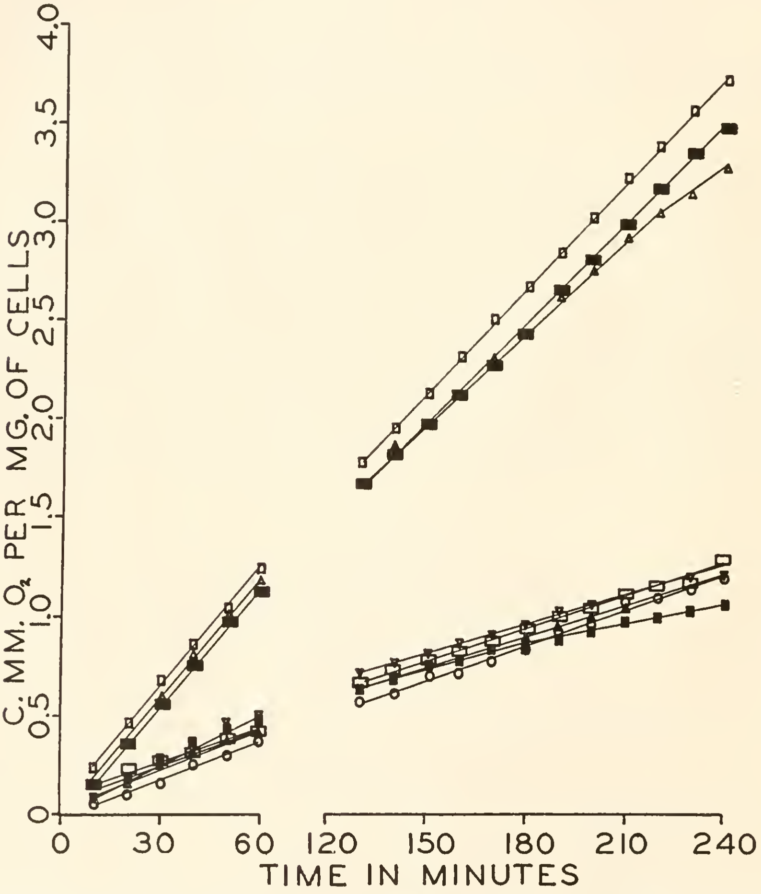

FIGURE 1. The effect of toxins on the oxygen consumption of unfertilized Arbacia eggs. Readings were taken for 60 minutes, then there was a 60 minute break and readings were again taken beginning at 120 minutes. □—*M. aureus;* ■—*Cl. tetani;* △—*C. diphtheriae;* ○—Sea water; ▽—*Cl. septicum;* ▲—*B. cereus;* ■—*Str. pyogenes;* ▭—*Cl. perfringens.*

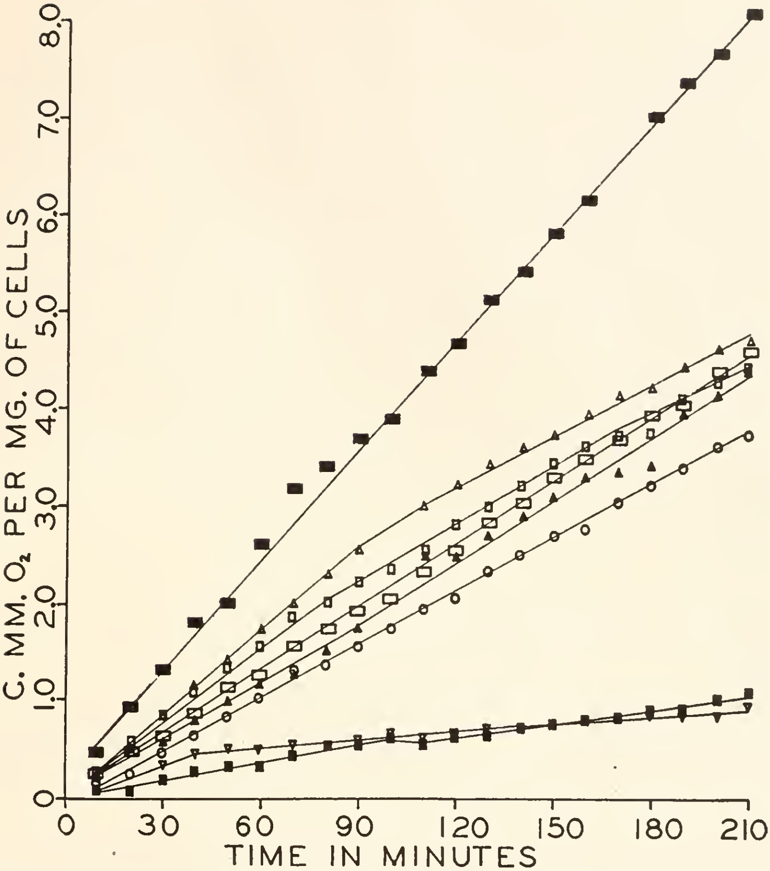

FIGURE 2. The effect of toxins on the oxygen consumption of unfertilized Asterias eggs. Readings were taken continuously for 210 minutes. ■—*Cl. tetani;* △—*C. diphtheriae;* ▭—*Cl. perfringens;* ☐—*M. aureus;* ▲—*B. cereus;* ○—Sea water; ▮—*Str. pyogenes;* ▽—*Cl. septicum.*

These data are not conclusive since the heated toxins apparently had an effect similar to that of the toxins. However, it is interesting to note that the effects of these toxins on the eggs are in many respects the same as those on erythrocytes. In the case of Arbacia eggs, the toxins from *C. diphtheriae, Cl. tetani,* and *M. aureus* accelerate respiration. The significance of the lack of inhibition by the streptococcal toxins will be discussed in a future publication. In the case of Asterias eggs, the tetanal toxins clearly accelerate respiration; the diphtherial and staphylococcal toxins may accelerate slightly for the first hour or two; the perfringens toxins may accelerate slightly, while there is a suggestion of a delayed acceleration in the case of the toxins of *B. cereus.* The streptococcal toxins and those of *Cl. septicum* inhibit respiration.

DISCUSSION

A comparison between these data and those previously reported (Hunter et al., 1949a, b) shows that in general the action of the toxins on the four types of cells studied is essentially the same. Marked acceleration of respiration was obtained only with micrococcal, tetanal and diphtherial toxins. The fact that in the presence of micrococcal toxins the respiration of chicken erythrocytes fell off, while this did not happen with the dogfish erythrocytes, may be explained by the hemolysis which occurred in the former case but not in the latter. In the presence of diphtherial toxins the initial acceleration is followed by a period during which there is no effect, or an inhibition of respiration of all but Arbacia eggs.

It is of interest to note that the rate of oxygen consumption of dogfish erythrocytes is considerably higher than that of chicken erythrocytes. Also, the sensitivity of the respiration of both types of cells to formalin suggests future experiments to study the respiratory mechanisms of these cells.

The relative resistance of the dogfish erythrocytes to the toxins containing lipid-splitting enzymes is worth noting. One of the most outstanding features of the action of toxins on the surface of chicken erythrocytes was the fact that the lecithinase in the toxins of *Cl. perfringens* and *B. cereus* and the lipase in the toxins of *M. aureus* markedly altered the chicken erythrocytes in a very short period of time. Much longer periods of exposure were required to alter the dogfish erythrocytes, particularly in the case of *M. aureus*.

SUMMARY

1. The toxins obtained from *Micrococcus aureus, Clostridium tetani* and *Corynebacterium diphtheriae* accelerate the rate of oxygen consumption of dogfish erythrocytes initially.

2. The toxins obtained from *Streptococcus pyogenes* markedly inhibit the rate of oxygen consumption of these cells after approximately one hour's exposure.

3. The toxins obtained from *Clostridium perfringens, Bacillus cereus* and *Clostridium septicum* have little effect on the oxygen consumption of dogfish erythrocytes.

4. The time for hemolysis of dogfish erythrocytes placed in 0.95 M ethylene glycol is decreased by exposure to the toxins of *Streptococcus pyogenes, Clostridium perfringens* and *Bacillus cereus.*

5. There is a suggestion that the toxins of *Cl. tetani* have a similar effect, but formalized tetanal toxins also decrease hemolysis times.

6. The time for hemolysis of dogfish erythrocytes placed in 0.95 M ethylene glycol is not altered by the presence of the toxins of *Clostridium septicum, Corynebacterium diphtheriae* or *Micrococcus aureus.*

7. The toxins of *Clostridium tetani, Micrococcus aureus* and *Corynebacterium diphtheriae* increase the rate of oxygen consumption of both Arbacia and Asterias eggs.

8. The toxins of *Clostridium perfringens* increase the rate of oxygen consumption of Asterias eggs but have little effect on the respiration of Arbacia eggs.

9. The toxins of *Streptococcus pyogenes* decrease the rate of respiration of Asterias eggs but have little effect on Arbacia eggs.

10. The toxins of *Bacillus cereus* have little influence on the respiration of either Arbacia or Asterias eggs.

11. The toxins of *Clostridium septicum* inhibit the respiration of Asterias eggs but have little influence on Arbacia eggs.

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RESPIRATION AND WATER LOSS IN THE ADULT BLOWFLY, PHORMIA REGINA, AND THEIR RELATION TO THE PHYSIOLOGICAL ACTION OF DDT

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I. Introduction

The interrelations of respiration, muscular activity, water content and content of respirable and irrespirable solid material have been little investigated in insects, particularly at different metabolic rates. The discovery that DDT enhances weight loss and oxygen consumption (Laug, 1945; Ludwig, 1946; Läuger et al., 1946; Buck and Keister, 1946, 1947) makes this insecticide a possible tool for such studies. At the same time, opportunity is afforded for further investigation of the still obscure physiological action of DDT.

II. Materials and Methods

Unless otherwise noted, two- to four-day old adult males of the bluebottle fly, *Phormia regina* Meigen, were used. Larvae were grown on horse meat, and adults given only sucrose, water and fresh orange *ad libitum*. All experiments were done at 25° C. In handling flies, brief carbon dioxide narcosis was used (Williams, 1946). This also reduced feeding variations by causing regurgitation of crop contents (up to 8 per cent of live weight). Flies were poisoned by walking for 10 or 15 minutes in a 1-liter bottle containing 250 mg. DDT deposited as a thick, continuous, tenacious microcrystalline layer which formed slowly from minute supersaturated droplets left after the solvent (5 cc. anesthesia ether) was blown out by 2 minutes' exposure to a strong air current. All doses used were lethal.

For weight-loss experiments, the flies were narcotized, sexed, sorted into four equal groups of 25 to 50, and weighed in cylindrical wire cages, 1 × 2 inches. Two groups were then poisoned with DDT. One poisoned and one control group were set up over 10 per cent NaOH ("Wet Control" and "Wet DDT"), and the remaining two over NaOH pellets ("Dry Control" and "Dry DDT"), in the jar chambers illustrated in Figure 1. The vapor pressure of water was about 19 mm. of Hg over the solution and about 1 mm. over the pellets. Since carbon dioxide was completely absorbed in both types of chamber, conditions were similar to those in the Warburg flasks (see below). Set-up time was about 15 minutes for controls and 32 minutes for poisoned groups. Additional flies were killed with cyanide vapor at the start of some experiments and dried to constant weight at 55–60° C. for initial dry weight percentage.

All four groups were without food, and ostensibly without drinking water, during all experiments, and incoördination made ingestion impossible for the poisoned flies in any case. However, the Wet Control flies might have obtained some drinking water if condensate appeared on the jar walls due to slight environmental temperature changes.

In the three types of weight loss experiments, flies were: (1) Removed briefly at intervals, weighed and replaced, thus giving the rate of gross weight loss. (2) Left for 10 hours, weighed, killed in cyanide vapor and dried to constant weight, giving the loss of dry weight (substrate) in 10 hours. (3) Left until dead, weighed, and dried to constant weight, giving the loss of solid matter previous to death.

Respiration was measured in 15–19 cc. Warburg flasks, one fly to a flask. High and low humidity were obtained by using, respectively, 0.33 cc. 8 per cent KOH, and solid KOH pellets as absorbents for carbon dioxide. The absorptive capacity of the moist surface of the pellets (240 mm^2) was at least as great as that of the 15 by 30 mm. ammonium-free filter paper fan in the "wet" flasks, and the risk of creepage was much less than with a comparable solution (120 per cent). Flies were segregated from the alkali by cylindrical screens of 50 mesh brass wire. As shown in Figure 2 the paper fan and pellets were kept from touching the screen, since contact of the brass with alkali produced an additional oxygen uptake. Removal of screens when apparent oxygen uptake was maximal showed that they did not significantly impede gas diffusion. In most experiments 10 poisoned and 3 control flies were used. They were weighed individually (while narcotized) to 0.1 mg. just before transfer to the flasks. Each flask was flushed with about 1400 cc. of tank oxygen, which had no consistent effect on uptake or behavior of either control or poisoned flies. Oxygen uptake and individual behavior were recorded every 15 minutes for 6 to 12 hours, then the flies were weighed, killed in cyanide vapor, and dried. Set-up time was 10 minutes for the controls, 25 for the poisoned flies. All

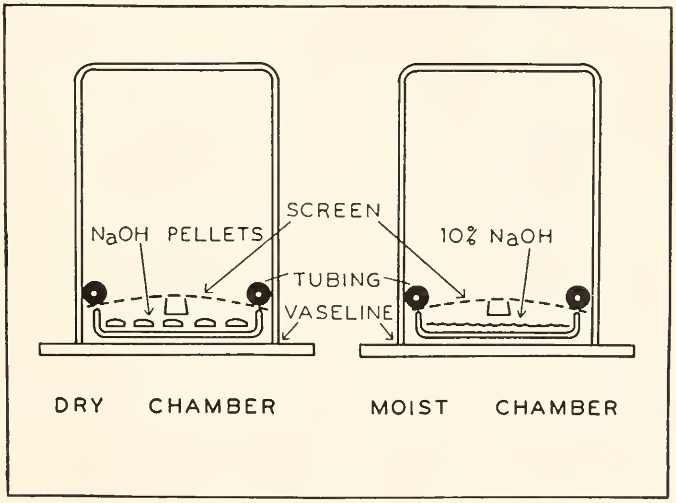

FIGURE 1. Gallon battery jars for use in following weight loss in dry and humid conditions. Flies' (upper) section separated from humidity-control section by circle of window-screen cut slightly larger than inside diameter of jar, and held between ring of pressure tubing and rim of 15 cm. petri dish. Handles attached to centers of screens.

gas volumes are expressed at S.T.P. Dry weights used in computing Q_{O_2}'s (mm^3/mg. dry wt./hr.) were taken as 35 per cent of initial live weights. Evidence validating various technical aspects of our respiration measurements is given in the Appendix.

Although it is usually assumed that carbon dioxide is the only gas liberated by insects, we ran six experiments (52 poisoned flies) in which half the flasks had 0.33 cc. of 5.5 per cent H_2SO_4 in the sidearms, in addition to alkali in the inset. The results indicated that no significant amounts of ammonia were produced by either control or poisoned flies in 5 hours. Likewise, in a number of experiments we interchanged flies between the "acid" and "non-acid" vessels without materially altering the apparent oxygen uptakes (Table IIB).

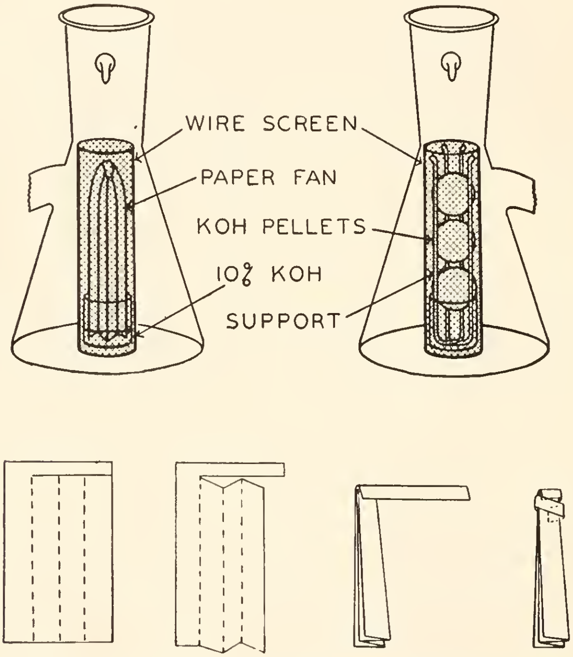

FIGURE 2. Warburg flasks modified for measuring oxygen uptake of flies under dry and humid conditions. The wires of the screening were actually horizontal and vertical rather than diagonal pellet supports made from glass rod. Other explanation in text.

Chitin was estimated by soaking weighed, hemisected flies in 8 per cent KOH for two to three weeks at room temperature, washing thoroughly, filtering on weighed paper, drying and weighing. This does not measure total skeletal material, since a large proportion of many insect cuticles is protein.

The individual variability encountered throughout the work, examples of which are seen in Tables II and III and in the standard errors indicated in Figures 4 and 9, made statistical validation of all conclusions desirable. Probability (P) values for the significance of differences between means were computed by "Student's" t test. The variability was presumably due to variations in age, feeding and dosage, though it was not materially reduced by applying the DDT in measured individual doses in kerosene solution. Coefficients of variation in weight within groups of 13 flies from a given batch of pupae were very reasonable (6–12 per cent), and an analysis of variance showed that, with flies of comparable age, variation between experiments was no greater than within. Tests of the influence of age and of narcosis time are described later.

III. Results

A. Weight changes in live flies

Weight loss was roughly linear in all four groups during the first 10 hours (Fig. 3) although there was a three-fold difference between the maximum and minimum group rates. Both groups in low humidity lost more weight than corresponding groups in high. The fact that the poisoned flies lost more than corresponding controls shows that DDT enhances weight loss. Overall weight loss after 10 hours becomes increasingly uncertain in poisoned groups, since some flies die, but it may considerably exceed the 30 per cent maximum found in 10 hours. In certain control groups, losses of 43 per cent occurred before death. Components of weight loss will be considered with the data on respiration and dry weight.

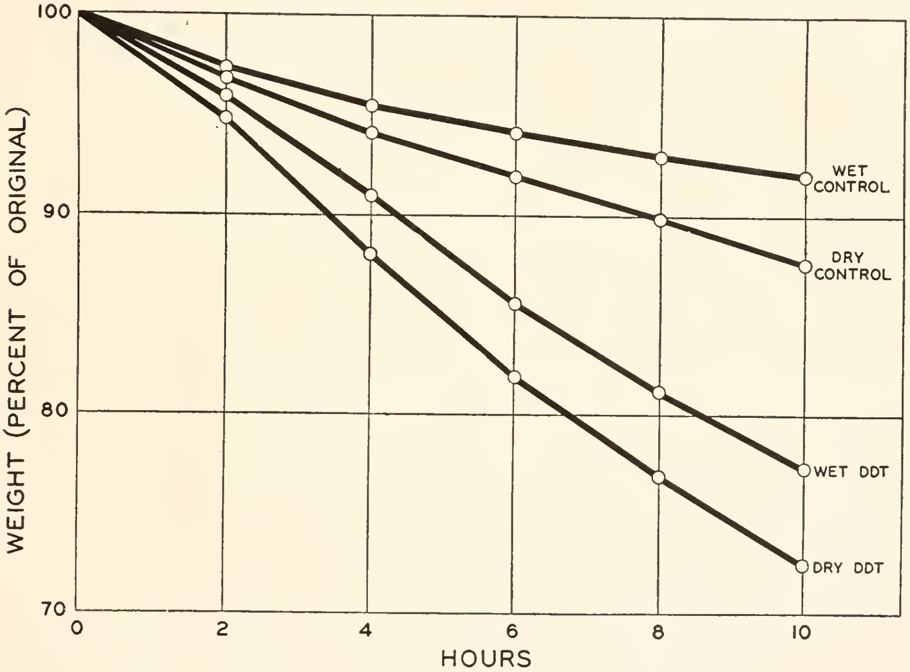

Figure 3. Live weight losses of normal and DDT-poisoned flies under dry and humid conditions. Each point represents the mean weight of 140 flies (6 experiments).

B. Rate of oxygen uptake of control flies

The oxygen consumption of individuals at rest was about 5 mm^3/mg. dry weight/hour (Fig. 4), a rate comparable to that in several other insects. However, occasional controls showed periods of uptake as great as those of poisoned flies (i.e. up to at least 10 times the usual control rate) coincident with periods of walking or running. Perturbations due to a single such individual out of twelve are seen in the Dry Control rate in Figure 4 (see also Fig. 7), which is otherwise not significantly different from that of the Wet Controls.

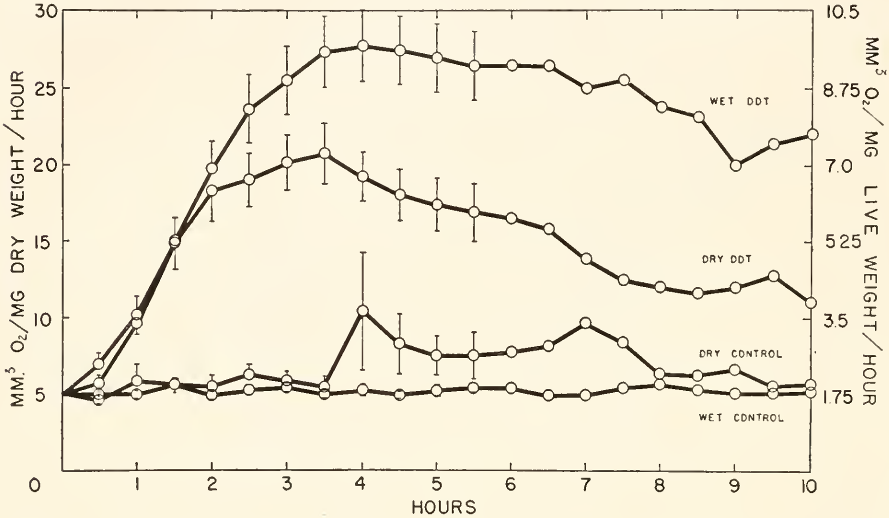

FIGURE 4. Oxygen uptake in normal and DDT-poisoned flies under dry and humid conditions (mean of representative series of 6 experiments). Thirty flies in each poisoned group, 12 dry controls, 6 wet controls. Cap lines from points indicate standard errors. The numbers of flies represented are 78 through 8 hours, 65 at 8½ hours, 51 at 9½ hours, and 35 at 10 hours.

C. Effect of DDT on oxygen uptake

The mean uptake of poisoned flies in a humid atmosphere rose steeply in about 3½ hours to over five times the control value, leveled off for about 3 hours, then declined gradually (Fig. 4). Some individuals showed brief early peaks 15–20 times the control level. The "dry" poisoned flies followed the same course for about 2 hours, but thereafter had very significantly lower uptakes ($P < 0.01$) whether calculated from initial weights or estimated weights at the times of measurement. That this is a true metabolic difference is indicated by the dry weight data given below (Fig. 5 and Table I) and by evidence (see Appendix) that oxygen uptake was measured with equal accuracy under wet and dry conditions. Accordingly, it can be concluded that a dry atmosphere partly inhibits oxygen uptake in poisoned flies. A similar inhibition has been reported in certain normal insects, but is there usually attributable to behavior differences. The unlikelihood that the effect is due to body temperature differences, such as have been observed in normal insects at different humidities, is indicated by the lack of effect in controls. Probably the explanation lies in the rapid depletion of body water in the "dry" poisoned flies.

D. Relation between oxygen uptake and weight loss

Figure 5 shows the relations between final live and dry weights (as percentages of original live weight) and total oxygen consumptions of individual normal and poisoned flies under dry and humid conditions. The individual variability, reflected in the remarkable spread in total uptakes, is not explained by the differing durations of the six experiments in this series (8½, 8½, 10, 10½, 11 and 12 hours).

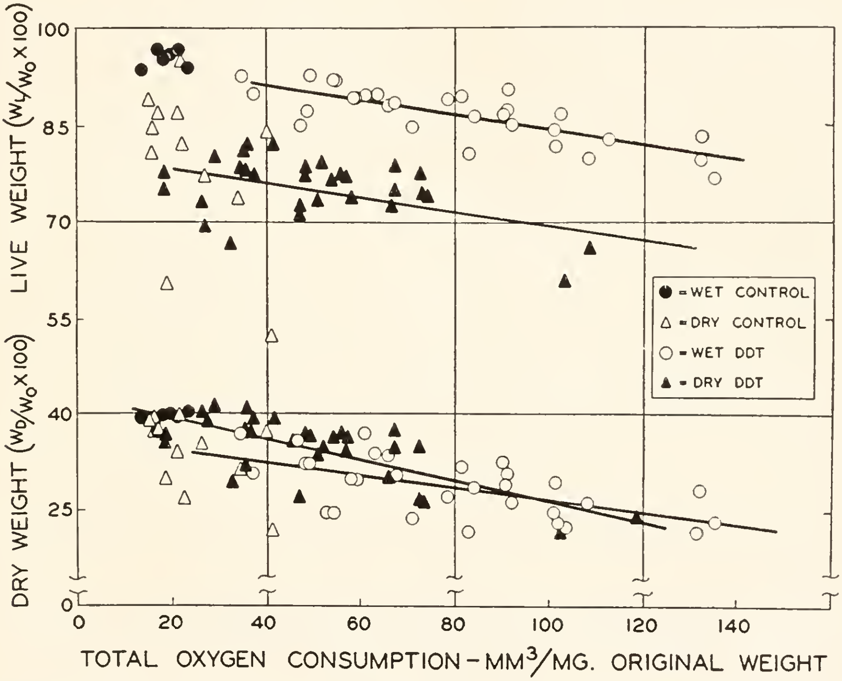

FIGURE 5. Relations between final live and dry weights (as percentages of original live weight) and total oxygen consumption in individual normal and DDT-poisoned flies under dry and humid conditions. Same experiments as those used in Figure 4. Lines fitted by method of least squares.

From Figure 5 we make the following deductions: (1) Loss of both live weight and dry weight of poisoned flies is roughly proportional to oxygen consumption. (2) The proportion of water to total solids changes little in the Wet DDT group. This means, however, that the respirable fraction of the total solid content decreases at a much higher rate than does water. (3) Total oxygen consumption is reduced by dry conditions. (4) Low humidity increases live weight loss. (5) Dry Controls seem to show a greater weight (water) loss in proportion to oxygen consumption than any other group.

E. Respiratory quotient

In experiments in which the insets of half the flasks contained 5.5 per cent H_2SO_4 instead of alkali, the net decrease in gas volume in the flasks with no absorption of carbon dioxide was almost negligible with both control and poisoned flies (Fig. 6 and Table IIA). The R.Q.'s calculated for the plateau period between 3 and 5½ hours were 0.90 for the control flies at rest, 0.93 for all controls, and 0.96 for poisoned flies. The figures indicate oxidation of a largely carbohydrate fuel. The situation is apparently different in poisoned Japanese beetle larvae, where Ludwig, by an undescribed method, obtain R.Q.'s of 0.6 to 0.8 and showed by analysis that much fat was metabolized.

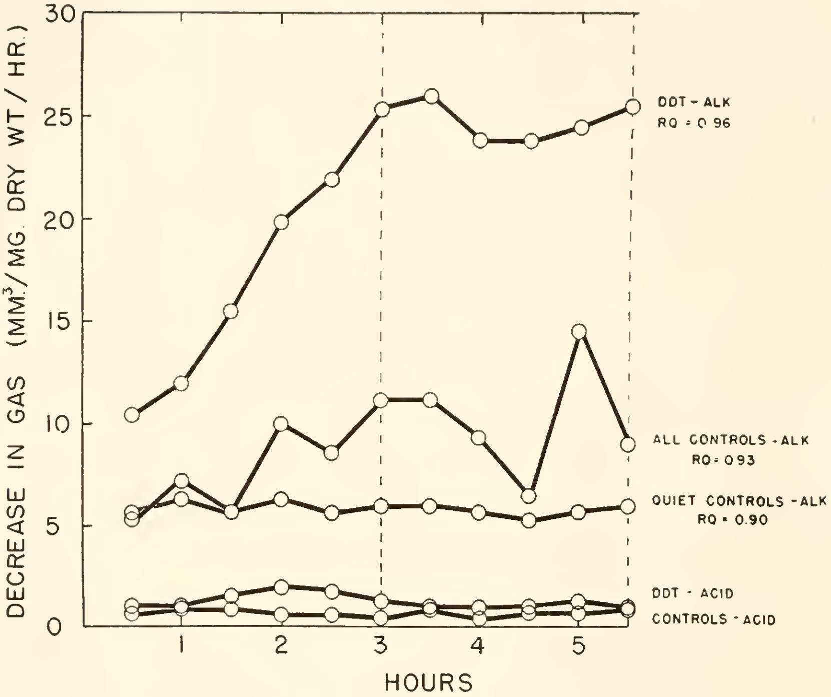

FIGURE 6. Net gas uptakes (decrease in gas volume) by normal and DDT-poisoned flies in respirometer flasks with and without absorption of carbon dioxide. Dotted lines indicate period used for calculation of respiratory quotient. Three experiments. Each DDT line based on 8 flies; alkali controls at rest, 3; all alkali controls, 6; acid controls, 9 (6 of which were at rest). Figures on 10 additional poisoned flies are given in Table IIA. The disturbing effect of activity on uptake is illustrated in the graph for all the alkaline controls.

F. Components and mechanism of weight loss

Since weight loss is nearly linear over the first 10 hours and is proportional to total oxygen consumption during the same period, rate of loss would be expected to be proportional to rate of oxygen uptake. However, uptake is far from linear in the poisoned flies, indicating that a breakdown of overall weight loss is desirable.

In Figure 7, the dotted line running through all the columns indicates the mean initial total solids (35 per cent).[1] The supposedly "irrespirable" fraction (11.9 per cent) is defined as the dry weight of Wet Control flies at death from starvation (minus the 2.5 per cent chitin fraction). The value for the "respirable solids", or material which can be used up in metabolism (20.6 per cent), was obtained by subtracting the weights of chitin and irrespirable solids from the total dry weight.

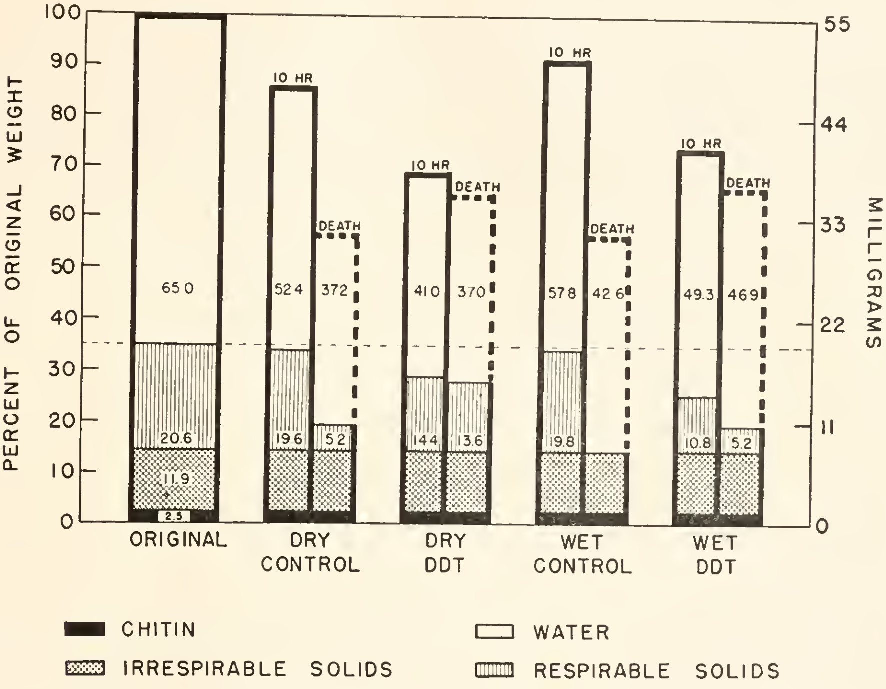

FIGURE 7. Content of water, respirable and irrespirable solids and chitin at 10 hours and at death of normal and DDT-poisoned flies in dry and humid conditions as percentages of original live weights. Based on measurements on about 860 flies in 9 experiments, including the 560 flies used for rate of weight loss measurements (Fig. 3). Water content at death subject to some uncertainty because flies did not all die simultaneously, and because the time of actual death was uncertain.

Figure 7 shows that live weights at 10 hours were in the order: Wet Control > Dry Control > Wet DDT > Dry DDT (in per cent of original live weights, 92.0 ± 1.30; 86.4 ± 1.42; 74.5 ± 1.39; 69.8 ± 1.92). All groups differ significantly ($P < 0.01$) except the Wet DDT and Dry DDT ($P = 0.08$). This indicates that both a dry environment and DDT enhance weight loss. Dry weights at 10 hours were in the order: Wet Control and Dry Control > Dry DDT > Wet

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[1] This figure increases slightly with age. This explains the fact that at presumptive zero oxygen uptake, the dry weights of the flies used in the wet-dry respiration experiments (Fig. 5) average nearer 40 per cent than 35, since all the flies in this eight-day series of experiments came from the same batch of pupae.

DDT (34.2 ± 1.47; 34.0 ± 1.30; 28.8 ± 0.64; 25.2 ± 0.33), the poisoned flies having reversed their relative positions. All groups differ significantly except the controls. The data agree with the respiration experiments in indicating that DDT enhances respiration (substrate loss), and that a dry atmosphere reduces this stimulation. Furthermore, they show that DDT promotes both water loss and substrate loss though not proportionately, and that water loss is greatest in the Dry DDT flies.

Table I

Interrelations of weight loss and oxygen consumption at 10 hours. Total oxygen uptakes (Col. 1) were estimated by graphical integration of the areas under the curves in Figure 4. Values in Columns 2, 3 and 5 come from the weight loss experiments (Fig. 6). Dry weights estimated from oxygen uptakes (Col. 4) were calculated on the basis of glucose, as were the weights of metabolic water corresponding to oxygen uptakes (Col. 7) and observed dry weight losses (Col. 8). Reserve water (Col. 9) was obtained by subtracting the means of Columns 7 and 8 from Column 3. "Metabolic water" in Column 10 is the mean of values in Columns 7 and 8.

	Mean total O_2 uptake/fly (mm^3)	Mean total weight loss/fly (mg.)	Mean total water loss/fly (mg.)	Mean total dry weight loss/fly (mg.)		Per cent water in total loss	Metabolic water loss (mg.) calculated from		Reserve water loss (mg.)	Per cent metabolic water in total water loss
				Calc.	Obs.		O_2 uptake	Obs. dry wt. loss		
	1	2	3	4	5	6	7	8	9	10
Wet DDT	4300	14.0	8.6	5.8	5.4	61	3.5	3.2	5.2	39
Dry DDT	2820	16.6	13.2	3.8	3.4	80	2.3	2.1	11.0	17
Dry Control	1300	7.5	6.9	1.7	0.6	92	1.0	0.36	6.2	10
Wet Control	960	4.4	4.0	1.3	0.4	91	0.8	0.24	3.5	13

Since, with the possible exception of the Wet Controls, the flies do not drink, it is possible to estimate the contributions of "metabolic" and "reserve" water to total water loss, on the basis of the observed R.Q. of nearly 1. As Table I shows, metabolic water estimated from oxygen uptake and that estimated from dry weight loss agree surprisingly well for the poisoned flies, considering the crudeness of the data and the fact that they were based on quite different sorts of experiments. The estimates for the controls are less reliable because of fewer flies in the respiration experiments and proportionately larger arithmetic errors in computing dry weight losses. However, the higher observed dry weight loss in the Dry Controls is consistent with the observation that this group was more active than the Wet Controls. Table I shows also that DDT increases both reserve and metabolic water losses, and that the former was several times as great as the latter. This fact plus the fact that water accounts for most of the weight lost (Col. 6) explain why the weight loss rates of the poisoned flies (Fig. 3) showed only a slight acceleration between 2 and 6 hours, when respiration increased several-fold.

Since poisoned flies did not defecate (see section on behavior), evaporation was the only significant avenue of water loss. The increased rate of loss might be associated with an increase in the normally low cuticular permeability but seems more likely to be due simply to increased transpiration, such as would occur if DDT caused the spiracles to remain open abnormally long. This would be consistent with the neuromuscular action of DDT, and also with the augmented metabolic rate. Unfortunately the abdominal spiracles of Phormia are too minute, and the great thoracic spiracles too inaccessible, for study without narcosis or artificial restraint. However, in the firefly *Photinus pyralis,* where the abdominal spiracles can be observed under relatively normal conditions, they open permanently in the stage of poisoning which corresponds symptomatically with the period of increasing respiration in Phormia (Buck, 1948).

Death occurred in less than one day in the average Dry DDT fly and more than eight in the Wet Controls. The water content of both Dry Controls and Dry DDT flies was around 36 per cent at death. However, the Wet DDT group still contained about 47 per cent water at death, hence death from DDT cannot be ascribed primarily to water loss.

As shown in Figure 7, the dry weights of flies which remained in the chambers until death were in the order: Dry DDT > Dry Control and Wet DDT > Wet Control (in per cent of original live weights, 28.0 ± 1.00; 19.6 ± 0.69; 19.6 ± 0.69; 14.4 ± 0.42). All groups differ very significantly except the Dry Control and Wet DDT. The Wet Control thus showed the lowest weight, instead of the highest as it did at 10 hours, and the Dry DDT the highest, instead of the next to lowest as it did at 10 hours. Hence, poisoned flies, although respiring faster than controls early in the experiment, died before they had lost as much solid material. Consequently, general substrate exhaustion cannot be the primary cause of death, assuming that poisoned flies utilize the same materials as controls.

G. Influence of hypoxia on DDT poisoning

In poisoned *Musca domestica* in air, Laug observed that oxygen uptake attained a high rate in 2 hours, then fell off rapidly to zero, coincident with cessation of movement in the fly. We observed the same thing in Phormia. Calculation showed that the cessation of uptake coincided with the exhaustion of oxygen in the flasks. Thus when the flasks were flushed with air or oxygen, the flies resumed kicking, and oxygen consumption again rose (Fig. 8). Therefore hypoxia, like cyclopropane anesthesia (Merril, Savit and Tobias, 1946), delays but does not permanently inhibit the symptoms. The symmetry of the initial peak (Fig. 8) indicates that pO_2 becomes limiting at about 90 mm. (10 per cent). This concentration was found limiting to oxygen uptake in flying *Lucilia sericata* by Davis and Fraenkel (1940), and to wing-beat frequency of flying Drosophila by Chadwick and Williams (unpublished).

In flies reviving from hypoxia, oxygen uptake often exceeded the original peak (Fig. 8), but the total uptake in the second burst of respiration showed little if any increase over the first. Since renewal of alkali (and air) after the second burst did not raise oxygen uptake above the control level, the total of the two bursts (ca. 6000 mm^3) either represented the maximum uptake of which those individuals were capable or indicated that no marked oxygen debt had been incurred.

H. Dosage effect

Laug found that house flies given 2.5 μg DDT/fly showed an earlier and smaller increase in oxygen uptake than flies given 1 μg/fly. Similarly, Ludwig's graphs indicate that peak uptake following 5 per cent DDT was higher than with either 10 per cent or 1 per cent, and that total uptakes were in the order 1 per cent > 5 per cent > 10 per cent.

To evaluate dosage effects in Phormia, oxygen uptakes were measured in culture-mates given 10 minute and 30 minute exposures to a DDT surface. As Figure 9 shows, oxygen consumption rose equally in the two groups to about three times the control rate. Thereafter the more heavily dosed flies showed the lower uptake, the difference being highly significant ($P < 0.01$) after 3 hours. Therefore, since heavier doses ordinarily give a quicker and larger kill than lighter, excessive respiration is not the direct cause of death from DDT.

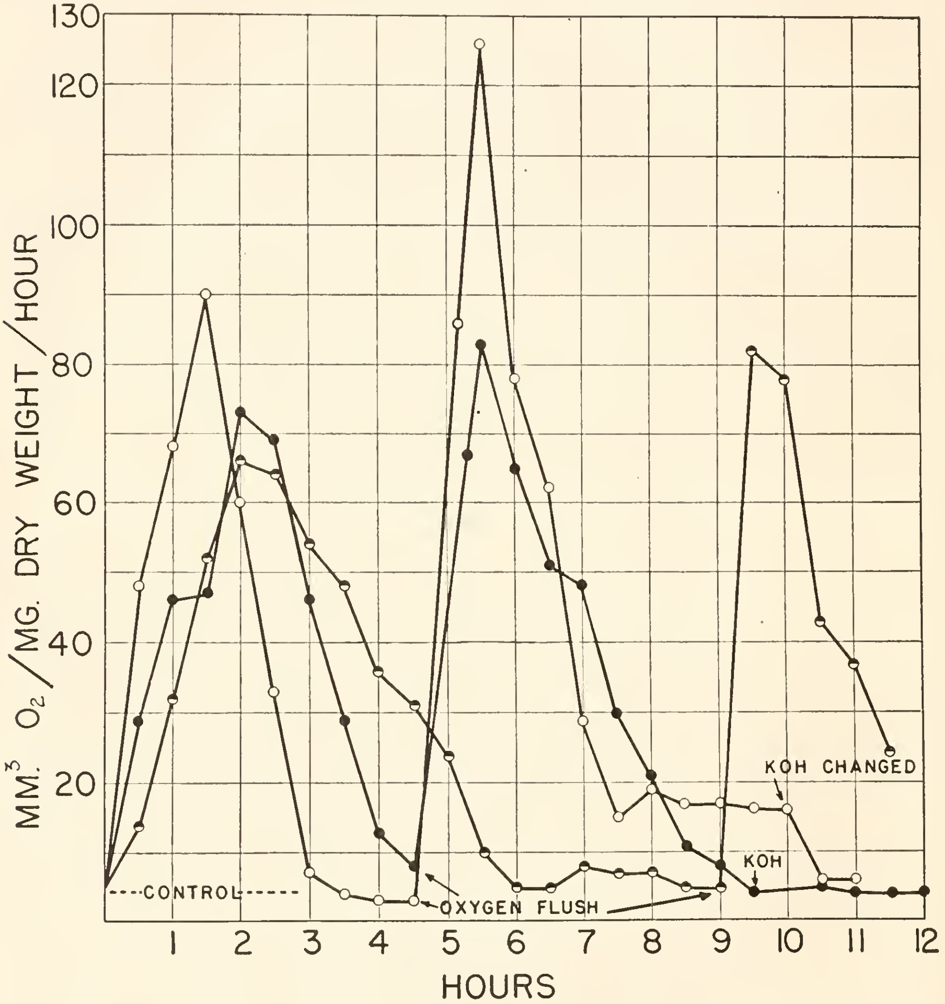

FIGURE 8. Oxygen uptakes of 3 flies respiring in air in Warburg vessels of about 15 cc. volume. Vessels flushed with oxygen at indicated points, and alkali renewed in 2 at indicated points.

I. Behavior of poisoned flies

The visible symptoms of DDT poisoning, though non-specific and individually variable, are of interest in connection with the origin of the enhanced oxygen uptake. Three indistinctly separated stages of poisoning could usually be recognized. The first, lasting 15 to 30 minutes, involves locomotor hyperactivity with progressive incoördination ending in prostration. In the second, lasting ½ to 1½ hours, the flies lie on their backs, exhibiting violent spasmodic extensions and flexions of the legs, with irregular movements of proboscis and wings. Occasionally the wing movements go over into a burst of furious buzzing which lasts a minute or more.

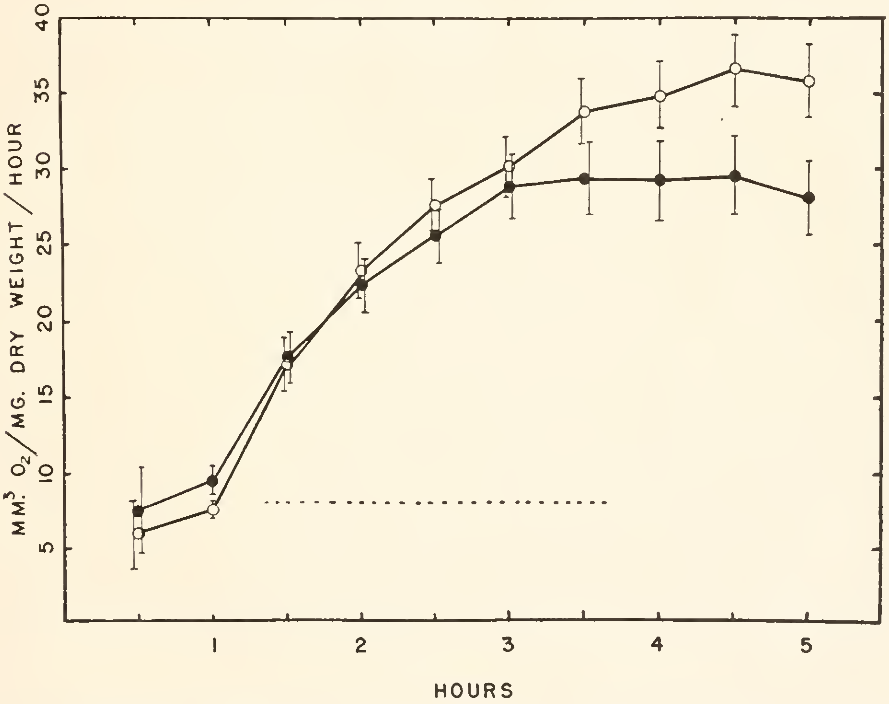

FIGURE 9. Oxygen uptake in flies given 10 minutes' exposure to DDT (open circles) and 30 minutes (closed circles). Cap lines on points indicate standard errors. Dotted line indicates control level. Upper curve 52 flies, lower curve 47 (11 experiments).

The third stage involves marked high-frequency tremors. The legs become more or less constantly flexed at the femoro-tibial joints, so that all six tarsi are brought close together. This stage continues for several hours, during which the tremors become less and less vigorous, and finally can be seen only under the microscope. At this point the fly usually voids a large drop of excrement (up to 4 mg.), the first since the start of the experiment. This adheres to the ventral surface of the abdomen and is included in the weight.

It is difficult to conclude whether or not the overt muscular activity corresponds well with the rate of oxygen uptake, although it would not necessarily do so if some muscles were in tetany. In accord with Laug's finding, prostration occurs before peak oxygen uptake is attained. Except for the brief periods of buzzing, when very high uptakes were recorded, the first two stages are usually past before peak uptake is reached. This is somewhat surprising because the kicking of Stage 2 appears to be the most violent sustained motor activity during poisoning. However, the gradually decreasing tremors during Stage 3 correspond well with the prolonged "plateau" period of oxygen uptake (Fig. 4), and the terminal, incontinent phase, where visible activity is minimal, is always associated with a very low uptake. Correlation of activity with uptake is also indicated by the fact that the flies given a 30 minute exposure to DDT in the dosage experiments showed less violent symptoms than those with a 10 minute exposure, and appeared to die sooner.

J. Hydrogen ion concentration in DDT poisoning

Another possible factor in the toxicity of DDT would be an acidosis from metabolic products accumulated during hyperactivity. We investigated this question crudely on breis made by blending groups of 50 flies in 35 cc. of distilled water for 2 minutes. Glass-electrode determinations on control groups kept 12 hours without food or water gave pH values of 7.00, 7.03 and 7.00. Similar determinations on poisoned flies, at a time when one-half to two-thirds of them had ceased motion (12 to 16 hours), gave pH values of 7.04, 7.13 and 7.13. All values are within the range of the bloods of many insects (Boche and Buck, 1942). The data indicate that there is no generalized acidosis during DDT poisoning.

K. Age and narcosis time as variables affecting the response to DDT

Numerous workers have reported that newly hatched or young insects are more susceptible to poisons than older insects, although the reverse seems to be true in some instances. To test the age factor, oxygen uptakes were measured in five-fly samples from the same batch of pupae at 4 and 8 days of age, and in nine-fly samples from two different batches (average ages 2 and 12 days). The results indicated clearly an earlier response and greater rate and total of oxygen uptake in the younger flies. This is somewhat unexpected in view of the finding of Williams, Barness and Sawyer (1943) that the glycogen content (and hence presumptive respiratory capacity) of Drosophila rises steeply during the first 7 to 10 days of adult life.

Oxygen uptakes of groups of 15 flies given 10 and 35 minutes' narcosis before poisoning were identical over 6½ hours. This indicates that the unavoidable slight differences in narcosis time in our experiments were unimportant. However, in flies exposed for 160 minutes to carbon dioxide before poisoning, the subsequent uptake was only slightly above the usual control level, and less than one-third that of companion flies given only 5 minutes' narcosis.

IV. Discussion

From the observations that anesthetics postpone the carbohydrate depletion (Merril, Savit and Tobias), the increased oxygen uptake (Läuger et al.), and the symptoms (Bodenstein, 1946) of DDT poisoning, it has been concluded that the increased uptake is due to the increased motor activity rather than to a specific stimulation of oxidative metabolism. This view is supported by our observations of a rough correlation between degree of activity and rate of oxygen uptake, and of high uptakes in active controls. Further evidence is afforded by the parallel between some of the physiological changes during poisoning and those during normal flight (for review see Chadwick and Gilmour, 1940; Williams, Barness and Sawyer; and Chadwick, 1947). Points of similarity are: (a) marked carbohydrate depletion, (b) R. Q. of about 1, (c) ten- to twenty-fold peak increase over resting oxygen uptake, (d) no well-defined oxygen debt, (e) oxygen becomes limiting to maximal activity at a tension of about 90 mm.

Ludwig's conclusion that carbohydrate exhaustion is the direct cause of death in poisoned Japanese beetles is opposed by the evidence of Merril, Savit and Tobias that roaches are not saved by glucose administration, nor by anesthesia sufficient to suppress symptoms and prevent carbohydrate depletion; by Chadwick's conclusion that normal Drosophila can survive for some hours after their carbohydrate has been exhausted; and by the present finding that flies dead from DDT poisoning have more "respirable substrate" left than starved controls.

V. Summary and Conclusions

Weight, oxygen uptake, and behavior were followed in normal and DDT-poisoned adults of the bluebottle fly, *Phormia regina,* under very humid and very dry conditions, with the following results:

1. Live weight loss was roughly linear over at least the first 10 hours. Poisoned flies lost more weight than unpoisoned, and "dry" groups more than corresponding "wet" groups. Water formed about 60 per cent of total weight loss in "Wet DDT" flies, and from 80 to 90 per cent in the other groups, and was probably lost almost entirely by spiracular transpiration. Metabolic water loss calculated from total oxygen uptakes was in reasonably good agreement with that calculated from dry weight loss. It is concluded that both DDT and a dry atmosphere enhance water loss; that DDT enhances loss of both metabolic and reserve water; and that loss of water is not the primary cause of death in DDT poisoning.

2. DDT induced an average five-fold increase in oxygen uptake of flies in a moist atmosphere. Individuals reached transient peaks of 15 to 20 times the control rate, associated with violent wing buzzing. The increase was significantly smaller in a dry atmosphere.

3. At the end of 10 hours, the dry weights of the four groups were in the order: Controls > Dry DDT > Wet DDT. At death the order was: Dry DDT > Dry Control and Wet DDT > Wet Control. It is therefore concluded that death is not primarily due to exhaustion of respirable substrate, assuming that the poisoned flies utilize the same materials as the controls. This conclusion is also supported by the fact that a heavier, more toxic dose of DDT produced a smaller increase in oxygen uptake than did a lighter.

4. Over a 10 hour period total oxygen uptake was proportional to both live and dry weight losses in the poisoned flies. In the Wet DDT flies, relative solid content remained approximately constant, and respirable substrate decreased at a higher rate than did water.

5. The estimated respiratory quotient was about 0.90 in controls, 0.96 in poisoned flies. Oxygen became limiting to the enhanced uptake, and to hyperactivity, at a tension of about 90 mm. Active controls reached rates of oxygen uptake com-

parable to those of poisoned flies. Overt activity showed a rough correlation with rate of oxygen uptake. Several parallels between metabolism in normal flight and physiological manifestations of DDT poisoning are pointed out. It is concluded that the increased oxygen uptake in DDT poisoning is due to the motor hyperactivity induced.

6. No ammonia production was found in either control or poisoned flies.

7. Hydrogen ion determinations on breis of normal and poisoned flies indicated that no general acidosis occurred during DDT poisoning.

8. The roles of dosage, age and narcosis in the variability of the results are discussed.

We are happy to acknowledge the advice of Drs. Edward Adolph, Leigh Chadwick, Edwin P. Laug, Kenneth Roeder, A. Glenn Richards, Julian Tobias and J. Franklin Yeager.

Appendix

Validative data on technique of respiration measurements, with tables concerning respiratory quotient, ammonia production and variability

1. Measurement of oxygen uptake at different humidities. Since oxygen uptake in Warburg flasks is almost always measured under humid conditions, it is necessary to show that no systematic physical error was involved in our measurements in a dry atmosphere. Calculation shows that the maximum possible error from the diverse ratios of water vapor to gas at the two humidities (which might produce a spurious excess in uptake in the more humid flask) is negligible. Heat of solution of the alkali pellets is likewise unimportant because no appreciable difference was seen in the apparent rates of oxygen uptake of dry and wet flies during the first two hours, when the effect should have been most pronounced, and no disturbance occurred in the Dry Control rate when moist outside air was introduced in resetting the manometers.

As an overall empirical check on our technique, we exchanged "wet" and "dry" flies at times when their rates of oxygen uptake were apparently steady (from 2¼ to 6½ hours after poisoning). In 25 flies transferred from "wet" to "dry" flasks, the mean rate of uptake in the half hour immediately following the change was 98.6 ± 4.0 per cent of that in the corresponding period previous to transfer. In 25 "dry" to "wet" transfers, the corresponding new rate was 98.3 ± 7.2 per cent of that prior to transfer. Similar results were obtained in comparing the hour previous to transfer with the hour subsequent to transfer. (The fact that uptake decreased after both exchanges merely reflects the usual gradual fall with time.) These results strongly indicate that our technique measured oxygen uptake with equal accuracy in high and low humidity, and that the difference in uptake at the two humidities is metabolic. Furthermore, it appears that the humidity factor, whatever its ultimate cause, operates only before the uptake plateau is attained.

2. Linearity and capacity of carbon dioxide absorption. In some experiments, apparent oxygen uptakes of over 2000 mm^3 per hour per 55 mg. fly were recorded for brief periods, and even the average plateau level for all the Wet DDT flies was over 500 mm^3/hr./fly (multiply the mm^3/mg. dry wt./hr. values given in the figures and tables by 19). Since these rates, and the total uptakes per fly over 8 to 10 hours, are far higher than those usually measured with the Warburg microrespirometer, it is necessary to show that absorption of carbon dioxide was not limiting in our experiments. In most of 12 instances in which two flies were put together after they had apparently reached their plateau phases of uptake (Table III) the resulting apparent oxygen uptake in the single vessel closely approximated the sum of the two previous individual uptakes (rates up to nearly 3500 mm^3/hr. were obtained). It consequently seems clear that the absorptive capacity of the alkali was adequate, as far as rate of production of carbon dioxide is concerned, and that the plateau period of uptake (Fig. 4) is not an artifact.

The calculated total carbon dioxide capacity of the alkali in the inset of the average flask was about 5300 mm^3. Although in the longest experiments (Fig. 4) the average total oxygen uptake was only about 4300 mm^3 in the most active group, several individuals approached, and (on the basis of an R. Q. of 1) a few even apparently exceeded the theoretical limit of carbon dioxide absorption. Accordingly, unless the R. Q. decreased late in the experiments (as might have happened, in view of Chadwick's finding of a great decrease in R. Q. of Drosophila after flight), it is possible that the oxygen consumptions recorded in the last hour or two of some of the experiments were actually somewhat lower than the true values. In a few flasks in which the alkali was renewed, uptake increased, but in others (for example some of those shown in Fig. 8 and Table III), it did not. Thus some individuals show a decrease in oxygen uptake like that shown in the average curve in Figure 4, even when there are no known technical limitations

TABLE II

Oxygen uptake in paired individual flies before and after exchanging experimental conditions. Data used in calculating R. Q. and evaluating inhibition by accumulating carbon dioxide (Part A) and in testing ammonia production (Part B). In part B "decrease in gas volume" is equivalent to "oxygen uptake." From 15 to 30 min. were lost in each transfer and re-equilibration.

Expt.	Fly	Original treatment	Rate of decrease in gas volume in successive ½ hr. periods before exchange (mm³/mg. dry wt./hr.)	Time of exchange (hr.)	Rate of decrease in gas volume in successive ½ hr. periods after exchange (mm³/mg. dry wt./hr.)	Rate of decrease in gas volume in successive ½ hr. periods after return to original state (mm³/mg. dry wt./hr.)
A. Exchanges between flasks with CO_2 absorption ("KOH") and those without ("acid")						
166	3	KOH	23 40 56 63	2¾	0.5 1.3 1.2 0.3	52 31 15 23
	4	Acid	0.5 0.5 0.7 1.1		9 10 12 11	0.1 0.8 0.8 0.1
	5	KOH	33 31 33 33	5	1.1 1.2 1.1	32
	6	Acid	1.0 0.7 0.9 0.7		14 18 21	0.7
	12	KOH	46 38 32 36	3¾	0.6 1.2 1.1 0.8	15 21 30
	13	Acid	1.3 1.5 1.0 1.0		19 18 16 13	0 0.4 0.8
168	3	KOH	16 49 64 70	2¾	2.4 2.1	33 15
	6	Acid	1.0 0.9 1.3 1.2		22 25	0.7 0.9 0.9
	5	KOH	29 36 38 41	3	1.0 1.1 0.7	55 62 55
	11	Acid	1.2 1.6 1.5 1.2		41 41 36	0.4 0.7 0.5
B. Exchanges between flasks with and without acid in side arms						
164	6	Acid	31 43 69	2¼	37 42	38 36 34 32
	9	Non-acid	22 44 57		87 51	20 15 18
	13	Acid	11 28 53 56	2¾	56 47 44	41 34
	14	Non-acid	16 34 36 26		32 34 30	35 34
165	9	Non-acid	27 22 31 33	4¾	28 23 12	
	14	Acid	36 36 40 42		35 33 27	

TABLE III

Oxygen uptake of paired individual flies separately and after being combined in one respirometer flask to test linearity of carbon dioxide absorption. Note that the uptakes are rates per unit weight, and thus those in the last column should be expected to approximate the means of the individual uptakes of the two combined flies, rather than their sums. Table also shows variation in "plateau" uptake levels within and between flies, variation in individual responses with time, and equivalence of carbon dioxide absorption by pellets and solution. Expts. 151 and 152, wet-dry comparisons; 162 and 164, testing for NH_3*; 170–178, dosage. From 15 to 30 min. were lost in each transfer and re-equilibration.*

Exp.	Fly	Original treatment	Rate of O_2 uptake in successive ½ hr. periods before combining (mm³/mg. dry wt./hr.)				Time of combining (hr.)	Rate of O_2 uptake in successive ½ hr. periods after combining (mm³/mg. dry wt./hr.)			
151	4	Wet	16	12	11	12	6½	13	14	15	19
	6	Wet	16	15	17	14					
	5	Dry	9	11	14	9	6½	10	7	7	5
	7	Dry	13	14	19	21					
	9	Dry	9	8	8	9	8½	30	15	16	
	11	Dry	33	35	38	39					
152	6	Wet	25	23	20	20	5½	19	18	16	
	10	Wet	18	19	18	19					
	11	Dry	20	23	16	16	5½	22	10	6	4
	13	Dry	42	35	31	28					
162	2	Non-acid	36	37	29	34	6½	30	33	34	
	10	Non-acid	26	29	33	29					
164	2	Non-acid		40	56	46	1¾	27	27	26*	
	3	Non-acid		30	47	48					
170	3	10′ DDT	40	40	42	44	5½	46	41		
	12	10′ DDT	43	39	42	39					
172	7	10′ DDT	33	42	45	53	5½	46	43†		
	12	10′ DDT	40	44	42	43					
173	11	30′ DDT	19	33	45	52	3½	50	45*		
	13	30′ DDT	59	69	55	59					
175	9	30′ DDT	49	60	74	74	4½	86	33*		
	11	30′ DDT	38	41	46	52					
178	4	10′ DDT	57	44	46	49	7	45*			
	6	10′ DDT	76	72	74	79					

* These flies were subsequently separated, and after addition of new KOH and oxygen their individual uptakes were lower than before they were combined.

† Addition of new KOH and oxygen did not change uptake.

on measurement. Adequacy of carbon dioxide absorption is also suggested by the correlation between total uptake and weights of Wet DDT flies (Fig. 5 and Table I).

3. Estimation of respiratory quotient. The method of estimating R. Q. depends on average oxygen uptake being substantially the same in the two sets of flies. This requirement would not be met if the activity of the flies in the "acid" vessels was reduced by the accumulating carbon dioxide (calculated potential maximum of 7 per cent in 3½ hours). The possibility of extensive inhibition is minimized by the facts that the net uptake in the acid vessels did not change; that no difference in behavior was observed in the two sets of flies; and that no change was produced in one of the acid vessels by flushing with oxygen. In supplementary exchange experiments (Table IIA), 4 of 5 flies originally in acid flasks did indeed have considerably lower rates of uptake than their partners originally in alkali flasks. However, the R. Q. calculated from the acid and alkali periods of the flies originally in acid flasks is 0.95, which is so close to the 0.96 derived from the data given in Figure 7 that it seems legitimate to conclude that the estimated R. Q.'s are of the correct order of magnitude.

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GROWTH OF OYSTERS, O. VIRGINICA, DURING DIFFERENT MONTHS

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INTRODUCTION

Although the American oyster, *Ostrea virginica,* is one of the most common bivalves of our Atlantic and Gulf Coasts, very little attention was given by past workers to various aspects of its growth, despite the fact that this field offers a large number of unanswered problems. For example, Moore (1898) in his voluminous article on the oyster devoted less than one page to a discussion of its growth. Churchill (1921) also confined himself to a few general statements on the growth of oysters under different environmental conditions. Yet, an understanding of the growth of oysters is of undeniable importance not only from a purely biological, but also from a practical, point of view because the oyster industry occupies one of the leading positions among the fisheries of the United States.

Nelson (1922) was perhaps the first to study more or less systematically certain phases of the growth of oysters. He measured and weighed a large group of New Jersey oysters in April and August of 1919 and again in March of 1920 noticing the increase in size and weight between the measurements. Loosanoff (1947) and his associates observed the increase in size of oysters of different ages grown during a three-year period in Milford Harbor, Connecticut. The oysters were measured once a year—in late autumn—to show the increase in size.

None of the observations made thus far was directed to study the relative increase in the size and volume of the oysters during each month of the year. This article presents the results of such studies which were carried on in Milford Harbor, Connecticut.

WINTER OBSERVATIONS

Because it has never been satisfactorily shown whether New England oysters continue to grow during the hibernation period, experiments were carried on for three successive winters to give the needed answer.

Oysters were prepared for the experiments as follows: After the shells were cleaned of all foreign matter, the edges of the shells of each oyster were filed off to make it easier to notice new growth if any formed. The oysters were then individually numbered with small celluloid tags. Later the length, width, and depth of each oyster were measured with a vernier caliper reading to 0.1 mm. The length represented the greatest anterior-posterior dimension. The width was measured along the maximum dorso-ventral line, and the depth represented the maximum distance between the outer surfaces of the two shells.

For determining the volume of an oyster a modified Grave's (1912) method, consisting of measuring the quantity of water displaced by an oyster, was used.

The oysters were kept moist before immersion, to avoid a possible error in determining the volume, because dry shells usually absorb small quantities of water. The method was found simple but reliable, the measurements being accurate within one or two per cent.

The first experiment was begun with 80 three-year-old oysters. After the measurements were completed on December 7, 1944, the oysters were put into a large wire tray which was placed on the bottom of Milford Harbor at a depth of approximately 3 feet below the mean low water level. The water temperature on that date was 3.0° C. Soon after the tray was placed in the Harbor a layer of ice was formed and, therefore, the oysters could not be examined at frequent intervals.

The first examination was made on March 7, 1945 when the temperature of the water was still near 0.0° C. Examination of the edges of the shells showed that not in a single case was new growth formed. The final examination was made on March 20, when the water temperature was reaching 5.0° C., thus indicating that the end of the hibernation period was approaching. During this examination the length, width, depth, and volume of each oyster were re-measured and the data compared with those obtained for the same individual the preceding fall. Only three oysters died during the winter. The measurements of the living 77 oysters showed that they did not change in size or volume during the winter.

The second experiment was made during the winter of 1945–1946. A group of 120 oysters was placed in Milford Harbor at the beginning of the hibernation period and re-examined in March. All the oysters survived the winter but their shells did not increase in length, width, or depth.

The third and final experiment was conducted with 58 oysters between December 9, 1946 and March 14, 1947. In addition to the observations made during the two previous winters, the weight of each oyster was ascertained at the beginning and at the end of hibernation. The results of the March measurements showed that with the exception of one oyster which had part of its shell broken off, there was no change in length, width, depth, volume, or weight during the winter.

As a result of the observations made during three winters, we may conclude that in northern waters the shells of the oysters do not increase in size, volume, or weight during the hibernation period. However, our laboratory observations, which will be discussed in a later part of this article, showed that if the temperature of the water is kept well above the hibernation point, the oysters will continue to grow even in the middle of winter.

Observations During the Growing Period

The first experiments to determine the increase in the size and volume of the oysters during each month of the growing period were begun in the spring of 1944, but had to be discontinued in the middle of the summer because the new growth, which is almost as thin as cigarette paper and extremely brittle, broke off at the slightest touch. The experiments started in 1945 were also discontinued several months later for the same reason.

Finally, in 1946 the observations were successfully completed. On March 29, 1946, a group of 120 adult oysters was placed in a large wire tray attached to a float anchored in Milford Harbor. The float rose and fell with the tide but the position of the tray always remained approximately 3 feet below the surface of the water. Before placing the oysters in the tray they were individually numbered, measured, and their volumes determined.

The oysters were re-examined and re-measured at the end of each month; the last measurements were made during the last days of November when the water temperature was becoming low enough to induce hibernation. To keep the oysters out of the water as little as possible during the examinations they were handled in groups of ten, because such small groups could be measured and returned to the water within a few minutes. Also, by working with small groups it was easier to avoid breaking the shells.

Of the original group of 120 oysters, 109 were alive at the end of the experiment. The conclusions offered in this article are based upon the data obtained from these survivals. The ranges in length, width, depth, and volume of the oysters at the beginning of the observations were 68.2–107.5; 50.3–85.8; 22.5–40.0 mm.; and 40–104 cc. respectively. At the end of the experiments the ranges were 85.3–135.0; 66.5–107.6; 26.4–44.3 mm.; and 65–176 cc. The mean length, width, depth, and volume of the oysters for each month are given in Table I.

TABLE I

Mean with standard error of length, width, depth and volume of oysters at the end of each month during growing period of 1946, Milford Harbor

Month	Mean			
	Length in mm.	Width in mm.	Depth in mm.	Volume in cc.
March	88.2±0.766	65.4±0.519	30.0±0.314	63.7±1.438
April	88.7±0.799	65.6±0.554	30.0±0.319	64.8±1.519
May	93.8±0.823	71.0±0.604	30.0±0.319	65.7±1.518
June	98.1±0.886	79.4±0.766	30.0±0.319	69.0±1.531
July	103.1±1.001	82.5±0.763	31.0±0.309	73.6±1.675
August	106.0±0.95	83.8±0.788	32.1±0.329	84.1±1.893
September	109.6±1.034	86.0±0.786	33.2±0.352	93.0±2.243
October	110.4±1.06	86.3±0.8	34.1±0.37	97.2±2.464
November	110.8±1.084	86.3±0.8	34.6±0.365	99.3±2.596

In estimating the monthly increases of each variate, which represented the difference between the means of each two consecutive months, the total increase for the entire growing season was taken as 100 per cent, and the monthly gains were calculated in relation to it. The results are presented in Figures 1 and 2.

As already mentioned, the oysters of our area do not increase in size or volume during the hibernation period, which extends roughly from the beginning of December until the end of March. Monthly observations showed, however, that in April the shells of the oysters begin to grow, the mean increase in length for that month constituting 2.21 per cent of the total annual increment (Fig. 1). During May, June, and July the increase in length is most rapid, being 22.57, 19.03 and 22.12 per cent respectively. Thus, during these three months the oysters achieved approximately 63.7 per cent of their annual increase in length. The percentages for August, September, October and November were 12.83, 15.93, 3.54 and 1.77

respectively. As can be seen, the months of October and November contribute but little to the total annual increase in length.

An increase in the width of the oyster shells began in April, simultaneously with an increase in length, but terminated in October, a month earlier than the latter (Fig. 1 and Table I). It was extremely rapid in May and especially in June,

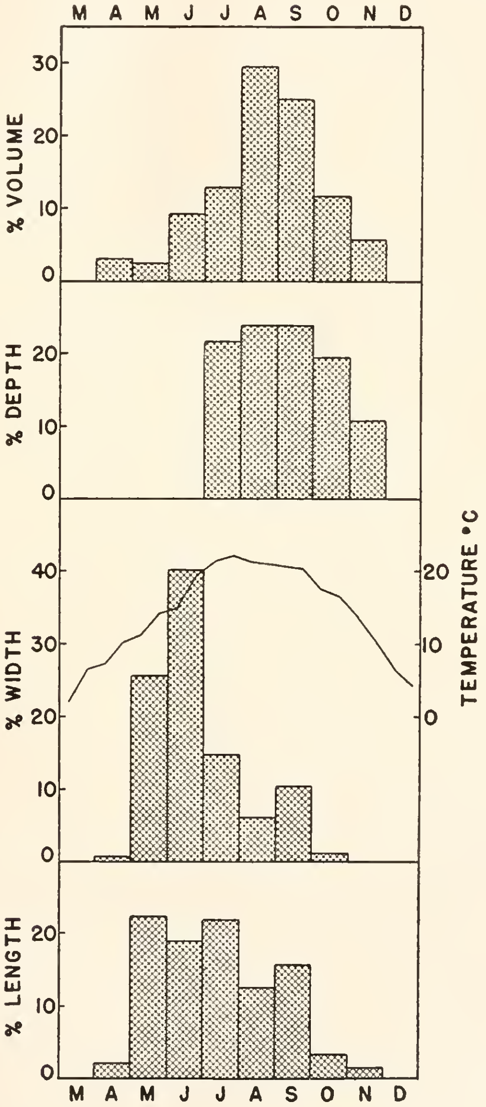

Figure 1. Per cent of increase in length, width, depth and volume of oysters during each month of the growing period. The total increase of each variate for the entire growing period, 1946 is taken as 100 per cent. Temperature curve is based upon semi-weekly records made at high water stages.

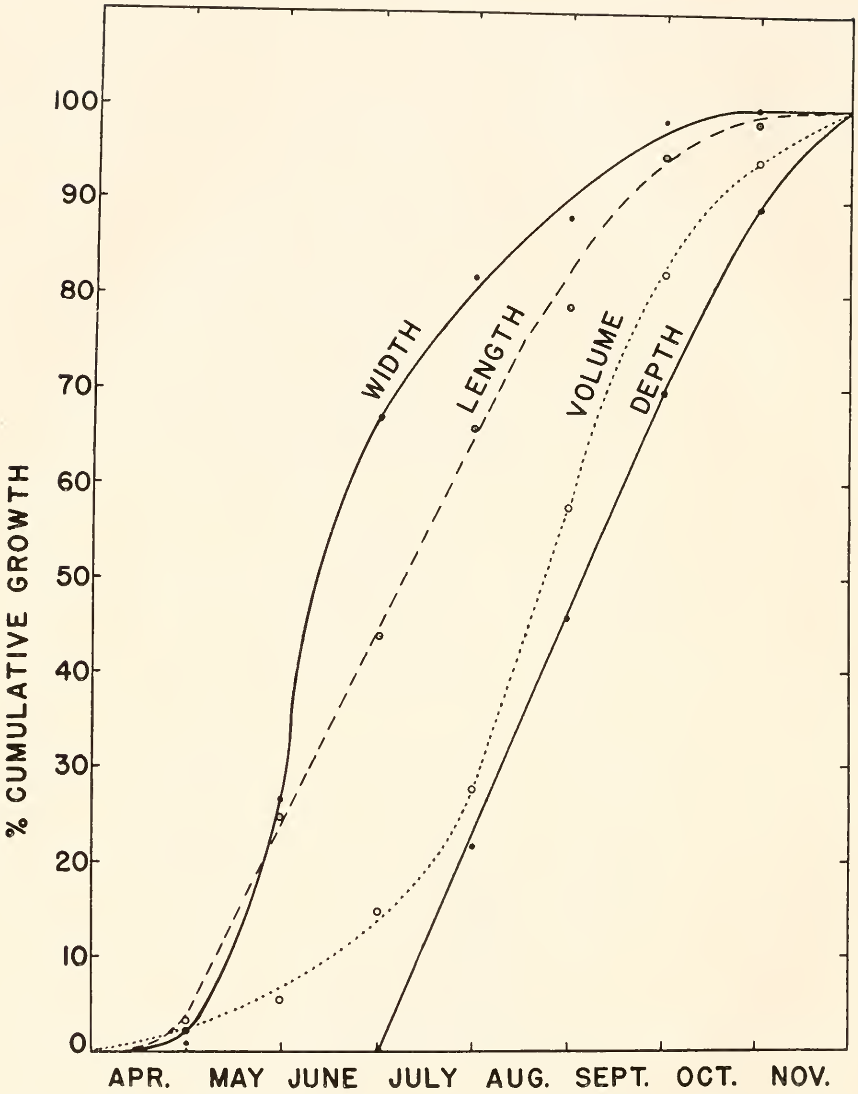

FIGURE 2. Per cent of cumulative growth in length, width, depth and volume of oysters recorded at the end of each month. The growth of each variate for the entire growing period of 1946 is taken as 100 per cent.

the latter month giving about 40 per cent of the total annual increase. In July, however, a sharp decrease was recorded. The decrease was even more pronounced during August.

Although the oysters increased in length, width, and volume during April, May, and June, the increase in the greatest depth was not appreciable until July (Figs. 1 and 2). During the first three months of the growing period the thinner parts of the shells became thicker, but this change was not reflected in the greatest depth, or thickness, of the oysters. Nevertheless, the observations on the increase in the greatest depth are of interest because they indicate, as do the studies of the increase in volume, that an increase in depth is largely achieved during the second part of the growing period.

The increase in volume of the oysters, the same as the increase in length, continued from April through November (Figs. 1, 2 and Table I). The greatest monthly increases were recorded during August and September, these two months giving approximately 55 per cent of the total annual increase in volume. As could be expected, the period of rapid increase in volume corresponded to that of greatest depth.

The marked increase in length and width of the oysters during May and June did not materially contribute to the increase in volume. This, of course, should be expected because newly formed shell-margins are very thin, displacing only small quantities of water.

Because the experimental oysters were individually numbered, it was possible to follow the increase in size of each individual from month to month throughout the entire growing period. This was done for two variates—length and volume. As usual, when working with a large number of animals, considerable individual differences were found. Nevertheless, the individual records showed the following interesting facts:

The maximum period during which oysters may grow in Milford Harbor is approximately of eight months' duration, extending from April to November, both months inclusive. However, only a small minority, comprising approximately 3 to 4 per cent of the entire group, grew during all the eight months, while for the majority of oysters the increase in length and volume was recorded only for five, six or seven months of the possible eight. About 3 per cent grew only three months and 10 per cent showed an increase in size for only four months, which were not always consecutive.

The chief increase in length and width of the oysters occurred during the first half of the eight-months' growing period, while the increase in the greatest depth and volume took place during the second half (Fig. 1). In this respect our observations are in agreement with those of Nelson (1922).

Not all the oysters began to grow in length and volume during the first month after the end of the hibernation period. Only about 48 per cent of the entire group increased in length in April, 49 per cent in May, while 3 per cent did not start growing until June. In volume, about 29 per cent began to show an increase in April, 40 per cent in May, 27 per cent in June, while 4 per cent did not show any increase until July.

Although it is true that we are usually concerned with the average animal, nevertheless, observations and records of unusually fast or slow-growing individuals are of significant biological value and interest because they may indicate that, within what may appear to be a homogeneous population, there may be distinct fast or slow-growing races. Some of the observations on individual oysters are given in the following paragraphs.

The greatest individual increment in length for the entire season was shown by oyster no. 22 which grew from 91.8 to 129.0 mm. in seven months, an increase of

37.2 mm. The smallest increase was shown by oyster no. 98 which grew from 77.6 to 85.3 mm., an increase of only 7.7 mm. This oyster grew in length only during two months out of a possible eight.

The greatest increase in volume for any individual was made by oyster no. 5, which increased from 104.0 to 176.0 cc., a total of 72 cc. in seven months. Oyster no. 17 showed an increase of only 8 cc., growing from 57.0 to 65.0 cc. in five months.

Individual records also made it possible to ascertain the maximum increase in length or volume of the fastest growing oysters for every month of the season (Table II). The largest monthly increase in length was made by oyster no. 90, which during July increased 15.2 mm., representing an increase of 17.9 per cent over the total length recorded at the end of June. During November the fastest growing oyster increased its length only by 3.4 per cent.

TABLE II

Greatest monthly increases in length or in volume shown by individual oysters. April–November, 1946. Milford Harbor

	April	May	June	July	Aug.	Sept.	Oct.	Nov.
Length								
Oyster no.	81	104	78	90	82	21	22	26
Old size in mm.	78.0	90.3	94.3	84.7	110.9	119.1	121.5	114.6
New size in mm.	82.5	100.8	105.6	99.9	117.9	129.1	129.0	118.5
Increase in mm.	4.5	10.5	11.3	15.2	7.0	10.0	7.5	3.9
% Increase	5.8	11.6	12.0	17.9	6.3	8.4	6.2	3.4
Volume								
Oyster no.	7	1	59	78	34	22	49	9
Old volume in cc.	73	78	73	72	79	98	106	128
New volume in cc.	78	82	84	87	99	127	122	136
Increase in cc.	5	4	11	15	20	29	16	8
% Increase	6.8	5.1	15.1	20.8	25.3	29.6	15.1	6.3

The greatest monthly increment in volume was made in September by oyster no. 22, which increased 29 cc. or 29.6 per cent over the volume recorded at the end of the preceding month.

It is significant that the greatest individual increases in length occurred during May, June, and July, that is, during the months when the group as a whole grew in length most rapidly. For the volume, both the greatest individual and group increments were noted in August and September (Fig. 1, Table II).

The records also show that the per cent of oysters increasing in length or volume varied considerably during different months. The increase was most common during July and August when almost all the oysters showed it, and the least noticeable in April and November.

Observations made on monthly growth of oysters in Milford Harbor suggest the following conclusions and deductions: Growth of the oysters, taken as a group, continued throughout the period extending from April to November, both months included, without definite interruption during the spawning season. This observation is in agreement with the conclusions of several investigators working with other lamellibranch mollusks. For example, Belding (1912) found that the hard-shell clam, *Venus mercenaria,* grows very fast during July and August when its spawning is in progress. During these two months the clams show approximately 45 per cent of the total annual increase in the length of the shell. Belding (1931) also found that the soft clam, *Mya arenaria,* which in Massachusetts waters spawns during June, July, and August, shows during these three months approximately 55 per cent of the annual increase in the length. Coe (1945, 1947) observed that the California bay-mussel, *Mytilus edulis diegensis,* also grows during the spawning period.

In other lamellibranchs, however, the rate of growth may be appreciably diminished during the spawning season. Belding (1910) noticed such a decrease in the bay scallop, *Pecten irradians.* Coe (1947) thinks that the decrease in monthly increments in length of the Pismo clam, *Tivela stultorum,* in August is due "to the requirements of the reproductive system and the successive acts of spawning."

In the case of oysters, Nelson (1922) found that *O. virginica* of the New Jersey coast grows rapidly until the spawning period but more slowly thereafter, while Orton (1935) observed two main periods of shell growth of *Ostrea edulis,* one in spring and one in autumn.

A very rapid increase in the mean length and width of the oysters of Milford Harbor occurred during May and June, i.e., during the period of most active gametogenesis for the oysters of this region (Loosanoff, 1942). Apparently, the process of development and accumulation of gametes did not interfere with the growth of the shell, at least as far as the increase in length and width was concerned. This conclusion is well supported by observations on oysters which are conditioned every winter in our laboratory to develop ripe eggs and spermatozoa (Loosanoff, 1945). The oysters are brought from the beds in the hibernating state and after being kept at room temperature for several hours are placed in trays with running warm water. In a month or less, depending upon the temperature of the conditioning trays, the oysters are ripe. Yet, during this period of extremely active gametogenesis the majority of the oysters grow rapidly in length and width, forming new shell-margins which quite often are over 1.0 cm. This proves, of course, that gonad development and rapid growth of shell may proceed simultaneously.

Mass spawning of the experimental oysters was observed during the last few days of June. There is no doubt that these oysters continued spawning during July and that many of them completed spawning during that month. The latter point was ascertained by opening Milford Harbor oysters not used in the experiment. Therefore, we concluded that since July was the month of most active spawning and since the increase in length during that month was very rapid, it is apparent that the spawning activities did not sharply affect the rate of increase in the shell length. In this respect our conclusions differ from those of Orton (1928) who reported that the rate of growth of the European oyster, *O. edulis,* is considerably slowed down during the breeding period.

It may be tempting to explain the slowing of the growth in width of our oysters during July by ascribing it to the spawning activities. Such an explanation, however, does not appear to be very conclusive because three other variates showed an increase during that month (Fig. 1). Even if the rate of increase in width during

July was considerably slower than that observed in June, it still was comparatively rapid, occupying the third position among the eight months of the growing season. Moreover, our laboratory experiments on the conditioning of oysters for spawning in the winter gave us additional proof that spawning does not stop, or seriously decrease, the rate of shell growth. For example, in February, 1949, a group of 105 oysters was brought from Long Island Sound, where the water temperature was below 5.0° C., and after being measured was placed in warm running sea water at 25.0° C. At the end of the ninth day at this temperature the oysters spawned. Some of the spawning oysters had already at that time a new shell growth which measured over 1.0 cm. After spawning, the oysters continued to form a new shell for some time.

The slowing down of the rate of growth in length and width during August also should not be attributed to the spawning activities because of the considerations presented above. Furthermore, spawning was almost completed during July. Perhaps the slow growth could be more logically associated with the post-spawning stage, during which emaciated oysters are, presumably, not in condition to divert much of their energy into building new shell substance. This assumption is again easily invalidated because of the pronounced acceleration in the increase in volume noticed in August and in early September (Fig. 1).

There are some indications of possible physiological antagonism between the growth of oysters and the process of accumulation of glycogen in their tissues, a phenomenon commonly known as "fattening of oysters." In our waters, chief accumulation of glycogen in the meats of oysters occurs between the completion of spawning and until hibernation, thus covering a period of approximately three months, namely, September, October, and November. During this period the rate of increase in size and volume of oysters progressively diminishes (Figs. 1 and 2). Whether this decrease is due to the true antagonism of the different physiological functions, or merely reflects the changes occurring in the surrounding water, remains at present undetermined.

The changes of the water temperature and the monthly rates of growth of oysters showed only a partial relationship. It is true that the increase in length and width of the shells recorded in April, May and June was accompanied by a steady rise in temperature (Fig. 1). In July, however, the rate of increase in the width markedly decreased, although the temperature remained above 20.0° C., but such a presumably favorable condition was not reflected in the rate of increase in length and width. The comparatively slow rate of growth in length and width observed during October cannot be explained by the unfavorably low temperature, because during that month the average temperature was not lower than that recorded for May and the early part of June when the shells grew so rapidly.

A much clearer relationship was found between the monthly increments in volume and the changes in water temperature (Fig. 1). In spring and early summer the monthly increments increased simultaneously with the increase in temperature. The period of the most rapid monthly increases in volume roughly corresponded to the period of maximal seasonal temperature, while in the fall both showed a gradual decline.

In connection with these studies it was thought desirable to determine by experimental means the rate of growth of groups of oysters kept at different temperatures. This was done in the winter time because it was easier then to maintain in the laboratory the desired temperatures merely by mixing definite quantities of cold and warm running sea water.

The warm sea water system, which is operated in our laboratory during the cold season, is regulated by a series of thermostats which control the temperature of the outflowing water. The temperature of our cold water is also very uniform. Therefore, in the winter time water of any temperature within the range of about 5.0° to 35.0° C. can be had by using constant level jars of cold and warm water and by regulating by stopcocks the flow from these jars into a mixing chamber until the desired temperature is obtained. From the mixing chamber the water is flowed into the trays or aquaria containing the oysters.

In the middle of February a shipment of four-year-old oysters, consisting of individuals of approximately the same size, was brought from the beds of Long Island Sound and placed for several hours in sea water of about 8.0° to 9.0° C. to let the oysters come out of hibernation. Then they were divided at random into four groups each containing 105 animals. After determining the average length and width of each group (Table III), the oysters were placed in trays with running water the temperature of which was brought up and then steadily maintained at approximately 10.0°, 15.0°, 20.0° or 25.0° C. All the trays were receiving the same quantity of water.

TABLE III

Average increase in length and width of oysters kept at temperatures of 10.0, 15.0, 20.0 or 25.0° C. from February 15 to March 16, 1949

Temperatures	10.0° C.		15.0° C.		20.0° C.		25.0° C.	
	L.	W.	L.	W.	L.	W.	L.	W.
Original measurements 2/15/49	92.1	70.3	91.5	68.6	92.5	70.1	94.0	70.1
Final measurements 3/16/49	93.4	71.3	99.9	76.0	100.1	77.3	98.5	73.2
Increase in mm.	1.3	1.0	8.4	7.4	7.6	7.2	4.5	3.1
% Increase	1.4	1.4	9.2	10.8	8.2	10.3	4.8	4.4

A month later the oysters were again measured (Table III). The 15.0° C. group grew best, showing at the end of the experiment an increase of 9.2 per cent in length and 10.8 per cent in width of shell. The maximum increase in length shown by the fastest growing oyster of this group was 21.0 mm. The growth of oysters kept at 20.0° C. was almost as fast as that of the 15.0° C. group. However, the 25.0° C. group grew much more slowly than the two above mentioned. and the 10.0° C. group showed only a slight increase in size. The maximum increase in length attained by the fastest growing oysters of the 25.0° and 10.0° C. groups was 14.0 and 5.0 mm. respectively.

In the 15.0° C. group all the oysters showed new growth and in 20.0° C. only one individual did not form new shell. In the 10.0° and 25.0° C. groups, however, many oysters did not grow.

Examination of the new shell growth showed that its character was different in the different groups. In the lowest group the new shell was, at the end of the experiment, still transparent, soft and flexible. In the 15.0° C. group, however, the new shell substance was already becoming harder and more brittle, and only the most recently formed part, confined to the edges of the shell, was still soft and flexible. This condition was even more pronounced at higher temperatures but, nevertheless, even in those groups many oysters were still forming new growth during the last days of the experiment.

Thus, under the conditions under which the experiment was run, the oysters grew most rapidly at temperatures of 15.0° and 20.0° C. Therefore, the optimum temperature range for their growth was either confined between these two temperatures or, what is more probable, extended a degree or two outside these two limits giving a range from approximately 13.0° to 22.0° C. It is interesting that the rapid increase in length and width shown in the spring and early summer by the oysters grown in Milford Harbor took place during May and June, in other words, when the water temperature was within the range given above (Fig. 1).

Our laboratory observations on the growth of oysters at different temperatures were, however, of too short a duration to find whether, if the experiment had been continued for several more months, the growth of each group would have proceeded at its original rate or would have shown some important changes. For example, it is possible that if the experiment had been prolonged, the rate of growth of the fast growing groups of 15.0° and 20.0° C. would have gradually decreased and, perhaps, eventually stopped, while the growth of the 10.0° C. group would have proceeded at the same or even at a somewhat faster rate than that shown during the first month of observation. It is planned to find the answer to this question in the near future.

With our present knowledge, it is impossible to estimate accurately the effect of food upon the growth of oysters. In a basin, such as Milford Harbor, where the tidal currents are swift and where the difference between high and low water levels may be as much as 9 feet, the quantity and quality of the material suspended in the water flowing over the oysters changes continuously. Even if it were possible to collect samples continuously, such samples would be of only limited value because many forms composing nanno and ultraplankton disintegrate almost immediately after collection. Thus, even if the quantity of material suspended in the water could be somehow determined, the quality of part of it would remain unknown.

Perhaps the greatest handicap facing the students of the role of food upon growth and other phases of the physiology of oysters is our lack of definite knowledge as to what really is the food of these mollusks. A full discussion on this subject is not the purpose of this article—those interested are referred to a summary published recently (Loosanoff and Engle, 1947). Briefly, however, while one school of investigators assumes that living plankton is the main ingredient of the oyster diet, the second school led by Coe (1945, 1947) is of the opinion that most of the nutrition of oysters, clams, mussels and other filter-feeding bivalve mollusks is derived from the intra-cellular digestion of particles of detritus originating from the disintegrated cells of marine animals and plants. Coe's conclusions appear to be well supported but, nevertheless, the issue is still debatable and not finally solved. As long as it remains in this stage, and as long as the value of different components of plankton and detritus are undetermined, it will remain impossible to formulate intelligently the relationships between the quantities or qualities of food present in the water over the oyster beds and the various aspects of the physiology of oysters or other mollusks closely related to them.

The difficulties of solving these problems are further complicated because oysters, and probably some other lamellibranchs, can feed efficiently only when the concentration of plankton (Loosanoff and Engle, 1947) or turbidity-creating substances as detritus or silt (Loosanoff and Tommers, 1948) do not exceed certain thresholds. If such thresholds are exceeded, the normal existence of mollusks becomes impossible. Thus, in addition to determining what organisms or materials constitute oyster food, it will also be necessary to determine their optimum concentrations in relation to the feeding and growth of oysters.

It should also be mentioned that a rapid increase in length and width of shell does not necessarily indicate that the oysters are growing under favorable conditions. For example, on several occasions at Milford Laboratory the oysters discarded after being used in the experiments were crowded in small aquaria through which only a trickle of water passed. Yet, within a short time some of them showed new shell growth. This growth was formed despite the fact that the oysters were not receiving enough food and that the water in which they were kept contained large quantities of waste products. Similar observations were made on oysters kept in heavy concentrations of micro-organisms, such as Chlorella and Nitzschia, which interfered with the normal feeding. The oysters eventually died (Loosanoff and Engle, 1947) but, nevertheless, even if their meats were emaciated, new shell growth was forming shortly before their death. These observations suggest that the factors involved in the growth of oysters are rather complex and at present not well understood.

The data and the conclusions on the monthly increase of oysters offered in this article are based upon only one year of observations. It is possible that during some years, when conditions are unusually favorable and the water temperature is considerably above normal during March or December, a slight increase in the size of the shells may be noted during these months. It is also possible that the monthly increases of the variates in different years would differ somewhat from those shown in our Figure 1. Nevertheless, it is believed that such variations would not basically change the trend of growth during the year.

Summary

1. The oysters grown in Milford Harbor did not increase in size, volume or weight during the hibernation period. However, if by some artificial means the temperature of the water is kept above the hibernation point, the oysters will continue to grow in the laboratory even in the middle of winter.

2. The maximum period during which oysters may grow in Milford Harbor is approximately of eight months' duration extending from April to November, both months inclusive. Only a small minority comprising approximately 3 to 4 per cent grew during all the eight months, while the majority grew only for five, six, or seven months. Some oysters did not start growing in length until June, and in volume until July.

3. The increase in length was most rapid during May, June, and July, representing 22.57, 19.03 and 22.12 per cent respectively of the total annual increment. The growth in width was especially rapid in June, giving 40 per cent of the total annual increase. The increase in the greatest depth was not appreciable until July.

4. The increase in volume continued from April through November; the greatest

monthly increases were recorded during August and September; these two months combined gave approximately 55 per cent of the annual increase in volume.

5. The increase in size was most common during July and August, when almost all the oysters showed it, and least noticeable in April and November.

6. The process of gametogenesis did not interfere with the growth of the shell, at least as far as the increase in length and width was concerned.

7. The spawning activities did not adversely affect the rate of increase in length and in volume.

8. The chief increase in length and width of the oysters occurred during the first half of the growing period, while the increase in depth and volume was most pronounced during the second half.

9. Changes in the rate of growth in length and width showed only partial relationship with changes in the water temperature. However, a rather definite relationship was found between the changes in the rate of increase in volume and changes in the water temperature.

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THE PREZONE PHENOMENON IN SPERM AGGLUTINATION

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The agglutination of the sperm of certain marine invertebrates by a substance known as fertilizin from the jelly hull of the eggs of the same species resembles serological reactions. This was first pointed out by Lillie (1919), and the concept has been expanded more recently by Tyler (cf. Tyler, 1948) on the basis of various features of the interactions of fertilizin and antifertilizin. One of these features is the occurrence of the zone phenomenon.

The zone phenomenon is typical of ordinary serological reactions (see Marrack, 1938). It is typified by the occurrence of maximum amounts of agglutination or precipitation when antigen and antibody are mixed in certain proportions. Increasing or decreasing the amount of antigen, or (in some cases) of antibody, above or below the optimum value results in decreasing amounts of agglutination or precipitation. The zones are also revealed by the occurrence of more rapid reactions (agglutination or precipitation) when the reagents are mixed in the proportions of the optimum zone than when mixed in other proportions. The mutual multivalence theory of antigen-antibody reactions proposed by Marrack (1938) and by Heidelberger (1939) offers an interpretation of the occurrence of zones. According to this theory, antigen and antibody molecules are both multivalent with respect to the mutually complementary groups by which they combine, with two or more such groups on each molecule. This would permit the formation of large aggregates of antigen and antibody. If the antigen were a cell surface, as in agglutination reactions, the process would result in agglutination. This hypothesis has received substantial support from the recent work of Pauling et al. (1944). Tyler (1940b) suggested that this line of reasoning could be applied to the agglutination of sperm by homologous fertilizin.

The occurrence of a prezone in an agglutination reaction may be interpreted in at least two ways according to the "framework" theory of antigen-antibody reaction. The prezone in agglutination reactions occurs in the region of highest agglutinin concentration; thus there would be such an excess of agglutinin molecules that the combining groups on the surface of the cell would each bind a separate agglutinin molecule, and no single agglutinin molecule would thus be likely to combine with more than one cell. This would result in little or no agglutination in very high concentrations of agglutinin and in increasing amounts of agglutination as the agglutinin concentration is lowered.

Another interpretation would be that "univalent agglutinin" molecules (agglutinin molecules with only one specific combining group each) are present along with the multivalent agglutinin molecules. Thus in regions of high agglutinin concentration, the univalent agglutinin would be able to combine with many of the specific combining groups on the agglutinogen surface and thus inhibit agglutination by the multivalent agglutinin. In any particular case, one or the other or both interpretations may apply.

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Tyler (1940a) noted the occurrence of the zone phenomenon, as exhibited primarily by rates of agglutination, when sperm of the limpet Megathura were mixed in various proportions with homologous egg water. With a given amount of sperm, for example, the most rapid agglutination does not occur with the strongest egg water but with lower concentrations. Further decrease in egg water concentration, of course, gives slower and less extensive reactions until the end point of no visible reaction is reached.

It was of interest to investigate the possible occurrence of the zone phenomenon in another group of animals, the sea urchins, which differs from the molluscs in certain details of the agglutination reaction of sperm with egg water (see Tyler, 1940a, 1941). Also it seemed desirable to examine this on the basis of degree of agglutination rather than simply on the basis of rate of reaction.

Materials and Methods

Sperm and fertilizin of the sea urchin *Lytechinus pictus* were used for the experimental work. The testes were carefully removed from the animals to avoid contamination with body fluid, and the "dry" sperm was allowed to extrude. This was filtered through bolting silk and stored in small sealed flasks at 8° C. until used. For testing purposes, a 1 per cent suspension of the sperm in filtered sea water was prepared. The fertilizin solution was prepared by allowing a thick suspension of eggs to stand for several hours, centrifuging it and using the supernatant, or by extraction with acid sea water as described by Tyler (1940b). No consistent differences were noted in the action of the fertilizin prepared by these two methods. For observing the agglutinating activity of the fertilizin preparations, serial dilutions in filtered sea water with a final volume of 1.0 ml. were prepared in 10 × 75 mm. test tubes, and 0.1 ml. of 1 per cent sperm suspension added. The tubes were agitated to mix the contents, and the degree of agglutination read macroscopically at 15 minutes after adding the sperm. The degree of agglutination was expressed in the customary 0 to 4 + terminology.

Experimental

In general, when constant amounts of homologous sperm are added to serial dilutions of fertilizin, the degree of agglutination observed is proportional to the fertilizin concentration. It should be pointed out that Lytechinus sperm does not show the rapid reversal of agglutination characteristic of the *Strongylocentrotus purpuratus* sperm plus fertilizin system. In the case of Lytechinus, the agglutination may persist for several hours, although this varies with sperm from different individuals. Extremely fresh sperm often shows a very low degree of agglutination when fertilizin is added, but after aging for several hours in the refrigerator, the degree of agglutination usually increases.

With some samples of Lytechinus fertilizin, it was noted that there was little or no agglutination in the highest fertilizin concentrations, although good agglutination occurred with lower concentrations. This resembles the prezone observed occasionally in a bacteria plus specific antiserum system, such as that described by Coca and Kelley (1921) for certain antisera against *Klebsiella capsulata*.

Wiener (1944) suggested that the prezone in the agglutination of Rh positive erythrocytes by certain anti-Rh sera was due to the presence of what he called "blocking antibodies," i.e., anti-Rh antibodies capable of specifically combining with Rh positive cells but unable to agglutinate them. The fact that the antibody had actually combined with the cells was established by showing that these cells could not be subsequently agglutinated by normal anti-Rh antisera.

It has been shown that treatment of fertilizin with heat, x-rays, and ultraviolet light (Tyler, 1941; Metz, 1942) converts it into the "univalent" form, that is, a form where it can no longer agglutinate the sperm, although it is still capable of combining specifically as may be shown by inhibition tests. This probably is due to the splitting of the fertilizin molecule into fragments containing only one effective group each.

In the first experiments conducted in the present work, the fertilizin was irradiated with ultraviolet light (source described by Spikes, 1944) to produce univalents. Later it was found that irradiating the fertilizin with visible light in the presence of a photo-sensitizing dye (eosin) according to the first method described by Tyler (1945) gave better results.

TABLE I

Degree of agglutination in the dilution of irradiated fertilizin indicated

	Titer of fertilizin									
Hours of irradiation	0	1	3	9	27	81	243	729	2187	6561
0	0	+++	+++	++++	++++	++++	+++	+	±	0
4	0	+	++	+++	+++	+++	++	+	±	0
20	0	0	+	++	+++	+++	++	±	±	0
26	0	0	0	+±	++	+++	++±	+	±	0
40	0	0	0	0	+	++±	++±	+	±	0
65	0	0	0	0	0	++±	+++	0	0	0

It was found that by using preparations containing univalent fertilizin along with the agglutinating type, the width of the prezone could be increased to a remarkable degree without appreciably affecting the beginning of the postzone. This is shown in Table I, which indicates the degree of agglutination resulting when constant amounts of sperm were added to serial dilutions of fertilizin irradiated with 750 foot candles of daylight-type fluorescent light in the presence of 0.2 per cent eosin for the number of hours indicated. If fertilizin which had been irradiated until it no longer produced any agglutination was added to normal fertilizin, a prezone was also produced.

Explanations for this induced prezone in terms of the "framework" theory of agglutination may now be suggested. If part of the molecules in a sample of fertilizin are converted into the univalent form by the irradiation, serial dilutions of this made, and constant amounts of sperm added to each dilution, there may be sufficient univalent fertilizin in the region of high fertilizin concentration to combine with all of the combining groups of all of the sperm present, and thus prevent agglutination. In setting up the serial dilutions the fertilizin solution is successively diluted, while the amount of sperm added to each dilution remains constant. Therefore, a dilution would be reached where there would no longer be sufficient univalent fertilizin to combine with all of the sperm, thus leaving some over to be agglutinated by the normal (multivalent) fertilizin present.

An alternative suggestion would be that the irradiation breaks the fertilizin molecule into a number of fragments which are still multivalent. Then when the sperm were added there would be such great competition for the combining groups on the sperm surface that it would be difficult for one fertilizin fragment to combine with more than one sperm. This again would result in a lower degree of agglutination in the region of high fertilizin concentration. There is at least one serious objection to this latter explanation, however. If the number of multivalent fragments was increased by the irradiating process, it would be expected that the endpoint of the agglutination reaction would occur at a higher dilution of the fertilizin than before the irradiation. An examination of the data in Table I, however, shows that the endpoint was moved in, rather than outward, to a region of higher fertilizin concentration.

From either of the above interpretations it could be predicted that multivalent fertilizin should be present in the tubes in the prezone region. This was shown to be true by successive absorptions with small quantities of sperm. The first few quantities of sperm added to the prezone dilutions were not agglutinated, but a point was soon reached where added sperm was strongly agglutinated. Presumably at this point (according to the first explanation suggested above) all of the univalent fertilizin was absorbed out so that the multivalent fertilizin present was able to combine with and agglutinate the sperm. The results of a typical experiment of this type are shown in Table II.

TABLE II

Degree of sperm agglutination in various dilutions of 65 hour irradiated fertilizin after successive absorptions with sperm

		Titer of fertilizin						
		0	3	9	27	81	243	729
Number of times absorbed with 0.2 ml. of 2% sperm	1	0	0	0	0	++±	+++	+
	2	0	0	0	++	+++	±	0
	3	0	0	±	++++	+	0	0
	4	0	+	++	++	±	0	0
	5	0	+++	+++	±	0	0	0
	6	0	+++	++++	0	0	0	0
	7	0	+++	++	0	0	0	0
	8	0	++++	0	0	0	0	0
	9	0	++++	0	0	0	0	0
	10	0	++++	0	0	0	0	0
	11	0	++++	0	0	0	0	0
	12	0	++++	0	0	0	0	0
	13	0	++	0	0	0	0	0
	14	0	±	0	0	0	0	0
	15	0	0	0	0	0	0	0

The work reported above is regarded as further evidence of the similarity of the reactions between the specifically combining substances of sperm and eggs to serological reactions.

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AN ELECTRON MICROSCOPE STUDY OF THE EGG MEMBRANES OF MELANOPLUS DIFFERENTIALIS (THOMAS)[1, 2]

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Extensive research has been carried out on the origin and the physical and chemical properties of the membranes surrounding the egg of the grasshopper *Melanoplus differentialis* (Thomas). The results of Campbell (1929), Jahn (1935a, 1935b, 1936), and Cole and Jahn (1937) seem most helpful in understanding its physical and chemical nature, while Slifer (1932, 1937, 1938a, 1938b), and Slifer and King (1934) give a clear picture of the structural relations of the egg membranes during embryonic development. It is the purpose of this paper to present results obtained from a study of the egg membranes with the aid of the RCA Electron Microscope, Model EMU-2B.

The outer membrane of the grasshopper's egg, the chorion (about $20\,\mu$ thick), is secreted by the cells of the maternal ovariole epithelium which enlarge during yolk deposition. Investigators seem to disagree regarding the formation and continuity of the vitelline membrane which lies just inside the inner surface of the chorion (Slifer, 1937). Since this membrane appears to become fragmentary as soon as embryonic development begins, it was not studied with the electron microscope.

At the time of laying, the egg, which has broken away from the ovariole epithelium, passes down the oviduct and out of the ovipositor into a pod made up of from 10 to 150 eggs. During the development of the blastoderm and its differentiation into germ band and serosa, very little change occurs in the egg membranes. The serosa cells migrate peripherally and completely surround the yolk and germ band by the fifth day (at 25° C.). They appear just inside the chorion as large, flat cells, with dense elliptical nuclei. During the sixth day, the serosa cells secrete on their periphery a non-chitinous (Campbell, 1929) membrane called the yellow cuticle (Jahn, 1935a, 1935b, and 1936). It is usually complete by the beginning of the seventh day at 25° C. Jahn (1936) found this thin membrane ($< 1\,\mu$) to show a high degree of ionic impermeability, and it may be closely related chemically to the cuticulin of *Rhodnius prolixus* (Wigglesworth, 1933).

The serosa cells also secrete a white fibrous membrane differing structurally and chemically from the yellow cuticle, and lying just inside of it. This layer, which gave Campbell and Jahn a positive chitosan test, is the white cuticle. Slifer's (1937) microscopical examination showed it "to be composed of innumerable fine threads tangled closely together." The deposition of this layered membrane (about $20\,\mu$ thick) requires one week at 25° C. (Slifer, 1937). She concluded, "The yel-

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[2] Aided by grant from the National Health Institute, administered by Professor J. H. Bodine.

low layer confers a high degree of impermeability; while the white layer is responsible for a greatly increased toughness and resistance to mechanical injury."

As the embryo develops, the chorion, if allowed to dry, cracks into an irregular pattern. The yellow cuticle is broken during the hatching process, but the embryo apparently is not strong enough to break the tough fibers of the white cuticle. Just before hatching, the latter, or the major portion of it, is digested away, making possible the emergence of the nymph. Slifer (1937, 1938b) has submitted evidence that the enzyme which is responsible for this digestion is produced by the pleuropodia.

The investigation of these membranes with the electron microscope requires special techniques and much patience. Since the electron beam is capable of penetrating tissue only about 1 μ in thickness, clear photographs of structures can only be made if the thickness is kept below 0.5 μ. There are many ways proposed for the preparing of extremely thin sections. All methods thus far noted in the literature fall into two categories: *A.* High speed microtomes, or *B.* Variations in the mechanics of sectioning.

Among the high speed microtomes which have been used and recommended are the "Cyclone Microtome" of O'Brien and McKinley (1943) and the two models by Fullam and Gessler (1946). One of the most difficult problems of the high speed microtome seems to be the locating of the sections after they are cut.

The earliest workers with the electron microscope tried many variations of geometry, mechanics, and chance. One of the methods, which involves a great deal of chance, is to cut the thinnest sections possible on the conventional microtome. These sections are re-embedded and re-sectioned, the operator hoping to obtain at least one very thin section out of many original cuts. The most commonly tried method, and the method used in this study, was the cutting of wedge-shaped sections on the conventional microtome as described by von Ardenne (1939), and Richards, Anderson and Hance (1942). From these a great many fine electronmicrographs have been produced. A very successful method used by Pease and Baker (1948) is the modification of the conventional Spencer Model 820 rotary microtome by decreasing the pitch of the diagonal backing plate to enable the cutting of 0.1 μ thick sections from doubly embedded material.

Another method of preparing sections, which has been used by Richards and his associates at the University of Minnesota, is to choose material which is naturally very thin. The results of these studies are reported by Richards and Anderson (1942), Anderson and Richards (1942), Richards and Korda (1947), and Richards and Korda (1948). The present investigator has employed this method in securing electronmicrographs of the yellow cuticle.

All membranes used were obtained from the eggs of animals kept under laboratory conditions as described by Boell (1935). To obtain the extremely thin sections of the membranes the following methods were used:

A. Fresh yellow cuticle. As stated above, the yellow cuticle is deposited by the serosa cells about the sixth day after the eggs are laid. Six-day-old eggs were placed in sodium hypochlorite solution to dissolve off the chorion (Slifer, 1945). The only membrane left enclosing the egg at this age is the yellow cuticle. The yellow cuticle of the egg was ruptured in isotonic saline solution, and a piece of it (after rinsing in isotonic saline solution and distilled water) is placed on the object screen of the electron microscope.

Plate I

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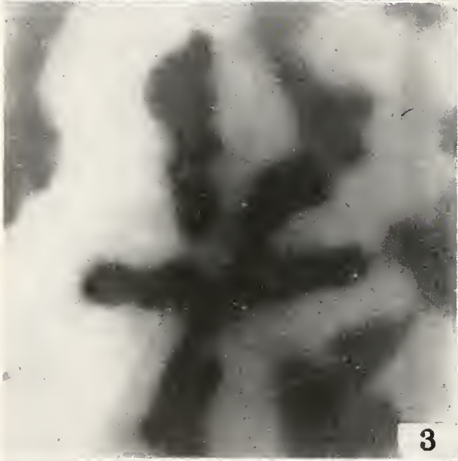
2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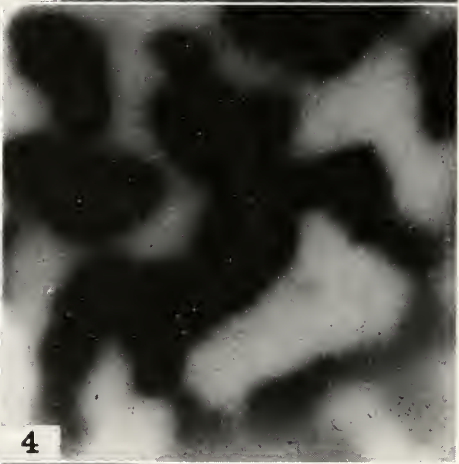
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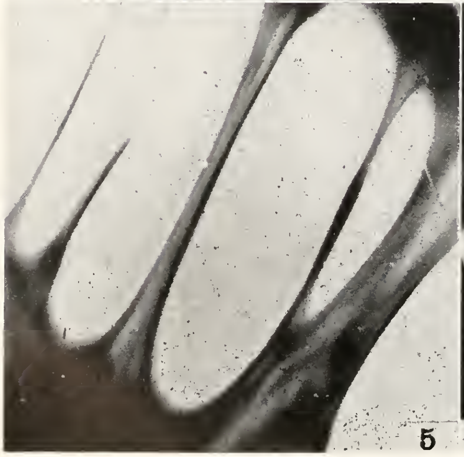
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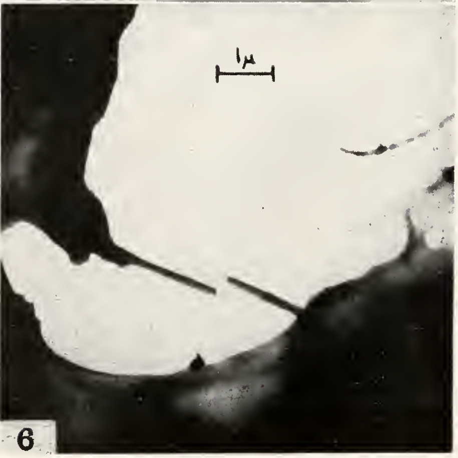

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B. Preserved sections of chorion and white cuticle.
  1. Geometrical method. One end of eggs at different stages of development was cut off, and the eggs fixed in Bouin and embedded in paraffin (56° MP) by the usual method. A number of eggs were embedded parallel in each paraffin block. The blocks were sectioned longitudinally or thereabouts in the conventional microtome at settings from 2–10 μ (Dempster, 1942). Some of the above eggs were prepared leaving yolk and embryo in the membranes, and some were prepared with the yolk and embryo removed before fixing. Little difference was noted in the results. The only sections having wedge characteristics were those cut from the eggs at the beginning and ending of the sectioning. These sections were placed in xylol to dissolve out the paraffin before being mounted on the object screen of the electron microscope.
  2. Modifying Spencer Model 820 Rotary Microtome. With this modification, eggs were doubly embedded as described by Pease and Baker (1948).

The placing of the sectioned material upon the object screen or grid near the center is not without problems. Since the grid wires are opaque, they always obscure a part of the material from view.

Since the limit of adjustment of the holder is only about five meshes of the grid in diameter, the exact centering of the specimen is critical. In sections carefully prepared and mounted, it may turn out that the material to be observed will have a location behind a wire of the grid. The super-drying of the specimen in the vacuum chamber, plus the "baking" it receives from the electron beams, renders the material so fragile and brittle that its relocation on the grid is next to impossible.

Results

An examination of the electronmicrographs with their titles and explanations reveals the structure of the membranes of the grasshopper egg. These figures are presented from the many pictures taken, as typical of the materials examined. In general, as the eggs become older, the chorion and yellow cuticle become electronically more dense. Thinner sections of older membranes were necessary before the material could be viewed or the electronmicrographs taken.

Figures 1 and 2 of the chorion (using wedge-shaped preserved sections) indicate that it appears to have no clearly resolvable internal structure. The shades of gray of the electronmicrographs vary greatly with the thickness of the sections. Figure 1 is a rather thick section of chorion, while Figure 2 shows a thinner section, and the additional thickness of torn yellow cuticle is at the edge.

Plate I

Figure 1. At edge of chorion from 14 days postdiapause eggs. Preserved specimen. × 13,000.

Figure 2. Chorion from 14 days postdiapause eggs. Torn edge of white cuticle at top. Preserved specimen. × 13,000.

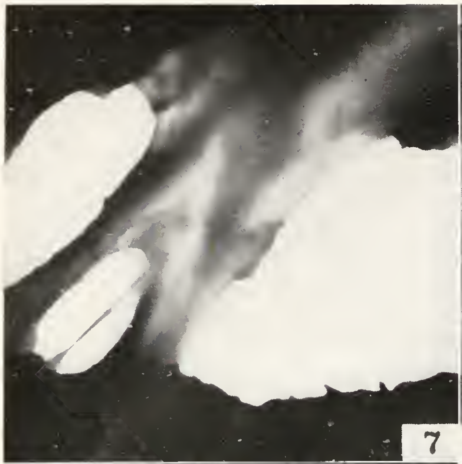
Figure 3. Yellow cuticle from 6-day-old eggs. Fresh specimen. × 13,000.

Figure 4. Same as Figure 3.

Figure 5. Stretched yellow cuticle from 6-day-old eggs. Fresh specimen. × 13,000.

Figure 6. Stretched yellow cuticle from 16 days postdiapause eggs. Preserved specimen. × 13,000.

PLATE II



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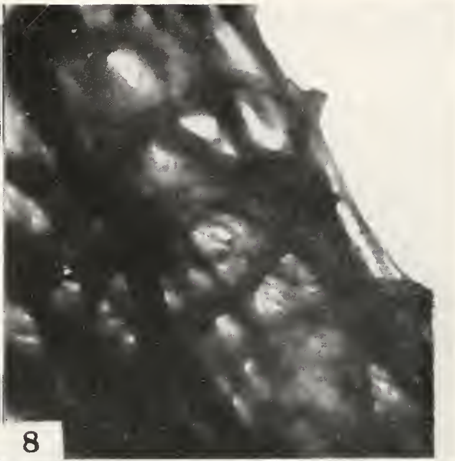

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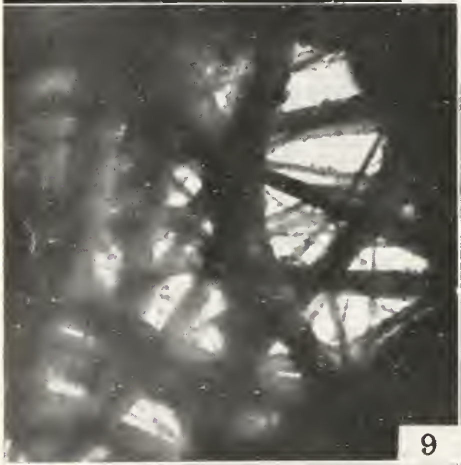

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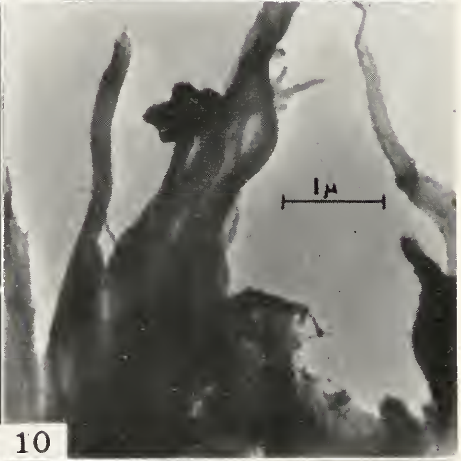

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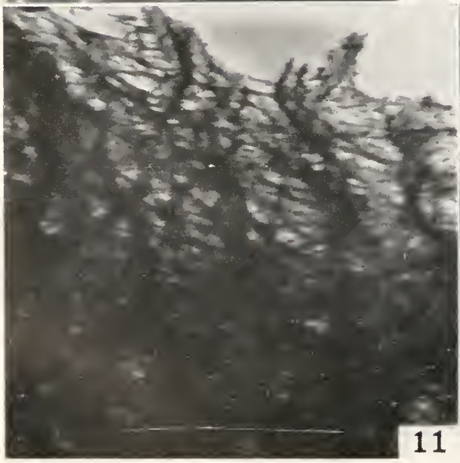

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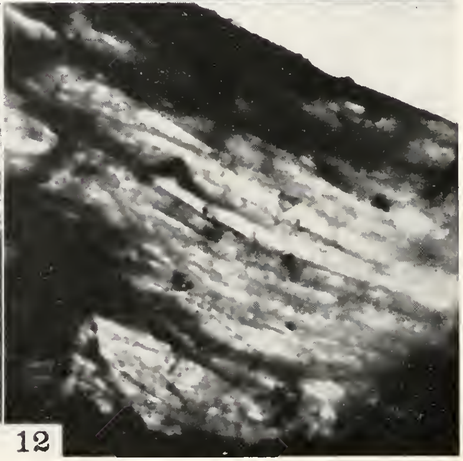

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Since wedge-shaped sections were used, no exact measurements of thickness were possible. All estimates or comparisons of thickness were the result of noting the distance from the thin edge of the wedge. Cross sections of preserved chorion, when stained with Delafield's hematoxylin and eosin or with Mallory's triple stain, appear under the high power oil immersion lens of the light compound microscope to be composed internally of short uneven fibers (Slifer, 1937, 1938a). However, the unstained, longitudinal, wedge-shaped sections of the chorion examined in the electron microscope seem to reveal no indications of a fiber-like structure. (See Figs. 1 and 2.)

Figures 3 and 4 of the yellow cuticle give evidence of the "minute ridges and tubercles" as described by Slifer (1937). These appear on the outer surface of this membrane. The ridges give greater thickness to the yellow cuticle and are believed to be responsible for the "Dalmatian-dog" pattern, which appears to be larger in the fresh material (Figs. 3 and 4) than in the preserved specimens (Figs. 13 and 14). This difference in size may be due to variations in material and shrinkage of the preserved material.

Figure 5 shows the results of stretching the fresh yellow cuticle which occurs as it dries in the electron microscope. Even in these stretched strands, variations in thickness are apparent. Figures 5 and 6 show preserved yellow cuticle which has been pulled to the point of breaking. Figures 5 and 6 both seem to indicate that there is stretching before the strands break. Note the blunt ends of the broken strands.

Figures 8 through 12 show the fibrous layered structure of the white cuticle. It was discovered that if the wedge-shaped sections were stained in eosin before being mounted on the grid in the electron microscope, the fibrous structure was largely obliterated. Since this was interpreted as an artifact, all stains were omitted on membranes employed in this study.

Decided differences of structure between the yellow and white cuticles, as evidenced in this and previous studies, point to the serosa cells as embryonic in nature and differing biochemically during development. The ability of the serosa cells to secrete two different structures or membranes, the yellow and the white cuticle within the same egg, indicates a similarity of function to the epidermal cells of insects (Wigglesworth, 1948).

Figures 8 and 9 show an extreme variation in the size of the fibers. There are indications of individual fibers and bundles of fibers both being present in the same white cuticle. No explanation is offered for the nodules on the fibers which are

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PLATE II

FIGURE 7. Stretched yellow cuticle appearing at crack in chorion from 16 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 8. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

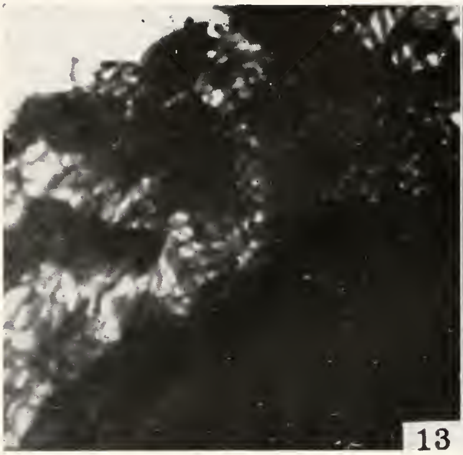
FIGURE 9. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 10. Stretched white cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 22,200.

FIGURE 11. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 12. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

PLATE III



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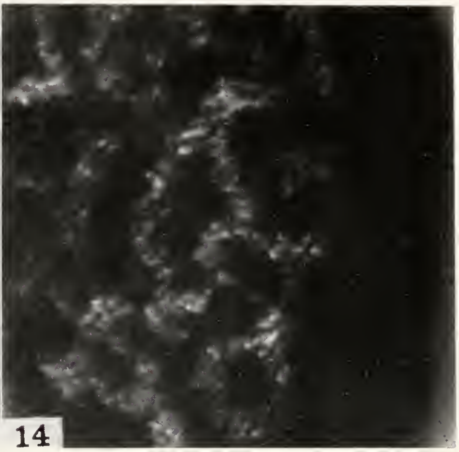

14

FIGURE 13. Yellow and white cuticle from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 14. Yellow and white cuticle from 11 days postdiapause eggs. Preserved specimen. × 13,000.

especially noticeable in Figure 9. Figure 10 shows the results of stretching white cuticle.

Figures 13 and 14 are combinations of yellow and white cuticle taken near the boundary of the two tissues. These electronmicrographs illustrate again the close proximity of the two cuticles, as parts of each may be viewed in one thin section. The "Dalmatian-dog" pattern is smaller in figures from preserved specimens than in Figures 3 and 4 from fresh yellow cuticle.

SUMMARY

Electronmicrographs of the grasshopper egg membranes by the methods used show that:

A. There is no clearly resolvable internal structure of the chorion.
B. The yellow cuticle has no clearly resolvable internal structure, but has varying thicknesses due to minute ridges or projections on its outer surface.
C. The white cuticle is fibrous in structure.

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PHOSPHATASES IN NORMAL AND REORGANIZING STENTORS

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The histochemical demonstration of phosphatase activity in *Stentor coeruleus*, although desirable in its own right, was undertaken primarily to determine to what extent such a study might amplify data obtained earlier (Weisz, 1949) with regard to metabolism and differentiation during normal and reorganizational stages in the life cycle of Stentor. Enzyme activity was studied in the normal, vegetative animal to provide a frame of reference, and this is compared with analogous data on starvation, regeneration, physiological reorganization, and vegetative division.

Experimental

In Stentor, phosphatases cannot be demonstrated with the usual "alkaline" techniques (e.g., Lillie, 1948). Air-dried (cf. below) or acetone-fixed test slides always yield precipitates in the same regions and in the same intensity as control slides prepared by omitting incubation or by substituting a calcium nitrate incubation for the treatment with substrate. Acid phosphatase activity, on the other hand, can be visualized.

The technique was adapted from Gomori's (1941) original method to demonstrate acid phosphatase activity. Stentors of appropriate stages were put on slides with a minimum of water, and after the organisms had been oriented, all excess water was drained off and the preparations were dried in a gentle air current from a fan. This method simultaneously flattens the animal to section thinness and fastens it to the glass. The preparations were then treated directly with the substrate.

In initial exploratory tests the usual method of fixation was tried by dropping living Stentors into chilled acetone before treating them with the substrate. It was found, however, that with this procedure much of the subsequent impregnation potential is lost, possibly due to partial enzyme inactivation by acetone (Stafford and Atkinson, 1948), possibly also due to a leaching out of some of the enzyme in the washing process (Barthelmez and Bensley, 1947). Air-drying on the other hand gave maximal and consistent results.

Of a number of substrates tried, sodium glycerophosphate was found to give the best results (cf. also Gomori, 1949), and this substrate, buffered to pH 4.7, and allowed to act for 15 to 20 hours at 25° C., was consequently used routinely. Post-incubation treatment followed the sulfide technique as outlined by Gomori. Control tests were carried out both by omitting incubation, and by poisoning the substrate with sodium fluoride (M/1000).

Recent work has raised some doubt whether the precipitates obtained by this method represent correct visualizations of phosphatase, and whether the loci of the precipitates correspond precisely to the *in vivo* sites of enzyme activity. Non-enzymatic impregnation of certain tissues by lead salts is known to occur (Lassek, 1947), especially during long incubation. The extent of this error can be estimated, however, by running controls in poisoned substrates. Inasmuch as in Stentor such control preparations do not reveal any precipitate, non-enzymatic impregnation probably does not occur to any appreciable extent under the present conditions of testing; the lead sulfide deposits obtained in the experimental material may thus be regarded presumptively as visualizations of enzyme activity. Nevertheless, in view of the possibility of enzyme shifting during the testing procedure (Barthelmez and Bensley, 1947), caution is warranted in interpreting the results, both with respect to the specificity and the localization of the reaction.

Examination of about 50 Stentors has shown that lead deposits are always found in a number of definite, circumscribed regions. In the ectoplasm, the deposits are centered in the basal granules of the body cilia and the membranelles. This gives the impression that the entire gullet and the peristome band are heavily impregnated, and that the longitudinal rows of body cilia are underscored with dark brown deposits. In the endoplasm, precipitates are particularly constant and abundant around the macronuclear nodes, but no deposits are observed within the nodes themselves. Heavy deposits are also found on the surface and probably within the endoplasmic vacuolar fat reserves (cf. Weisz, 1949), as well as in the immediate vicinity of the gastrioles. (In contrast, preparations fixed in acetone before incubation reveal only light deposits in the circumnuclear site, and no other part of the organism is impregnated.)

In starvation, a gradual decrease in the phosphatase reaction becomes manifest. Deposits in the fat vacuoles and near the gastrioles disappear first. By the time the oral area is about to be resorbed, only the regions around the macronuclei, and the basal granules of the membranelles, still yield a faint reaction (at this stage the preparations resemble those of normal animals which had been fixed in acetone). After the degeneration of the oral area even the circumnuclear activity soon disappears, and no part of the animal reveals any lead sulfide deposits. The data for physiological reorganization and vegetative division are rather parallel, and may be discussed together. The normal sites of activity largely persist unchanged throughout both types of reorganization. The point of interest centers around the regions in which new peristome bands are differentiated, anteriorly in physiological reorganization, and at mid-body in division (cf. Weisz, 1949). In every case, as in normal membranelles, newly differentiated membranelles show a high degree of activity in their basal granules. Such activity, however, can never be observed before the membranelles themselves have formed and are functional. In areas adjacent to newly formed peristome sections, i.e., in areas in which new membranelles will appear within a short time, activity is not yet evident.

Tests carried out on regenerating posterior fragments afford another opportunity to check on this point. Since the time at which new membranelles appear in a Stentor fragment is known (Weisz, 1948), it is possible to test for phosphatase before as well as after peristome new-formation. Such paired tests can be carried out on fragments obtained from the same animal. This was done, with results as above: presumptive sites of newly differentiating membranelles do not reveal any deposits; the latter become manifest only when the membranelles themselves can first be seen in an active state.

Apart from these differences in the presumptive oral area, regenerating fragments do not differ from normal intact animals in the extent and the localization of the sulfide deposits.

If the deposits may indeed be regarded as visualizations of acid phosphatase activity, these observations tend to throw some light on the function of the enzymes in the basal granules of the membranelles and the body cilia, even if only in a negative sense: if the enzyme were to appear just prior to membranelle formation, a role concerned with the mechanics of ciliary differentiation and structural maintenance might be tentatively ascribed to the enzyme. Since this, however, is not the case, the enzyme may possibly be involved in the energetics of ciliary motion.

In summary, the results tend to show that phosphatases in Stentor are fairly consistently present at definite loci of the cytosome, and that reorganization processes, unless they lead to the death of an animal or a fragment, are not correlated with significant changes in enzyme distribution. Newly differentiating organelles manifest characteristic enzymatic activity in parallel with morphological differentiation as such. The presence of phosphatases at circumnuclear sites may be significant in view of evidence (Weisz, 1949) that the macronuclear nodes discharge secretions (possibly phosphate-containing nucleic acid derivatives) into the endoplasm.

Summary

Phosphatase activity is studied in *Stentor coeruleus* by means of histochemical methods. "Alkaline" procedures are negative. Acid phosphatase may be consistently demonstrated in normal Stentors around the macronuclei, in the basal granules of the membranelles and the body cilia, in the endoplasmic fat vacuoles, and around the gastrioles. During starvation a gradual decrease in intensity and distribution of enzyme activity is observed, while in regeneration, physiological reorganization, and in vegetative division, activity remains unaltered in comparison to the normal animal. Presumptive evidence is obtained indicating that acid phosphatase in the basal granules is not primarily a factor in ciliary differentiation.

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THE PROTHORACIC GLANDS OF INSECTS IN RETROSPECT AND IN PROSPECT

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In 1762 the French anatomist, Lyonet, described as "granulated vessels" a pair of minute organs located within the thorax of caterpillars. This description was soon forgotten and for 187 years has been buried among the literature pertaining to insect anatomy. Meanwhile, within the twentieth century, the very same organs have been, and apparently continue to be, rediscovered by various investigators.

The resurrection of Lyonet's description of the "granulated vessels" seems particularly appropriate at the present time. For, within the past ten years, it has become increasingly evident that these organs, now known as "prothoracic glands," are among the most important endocrine glands in insects. The following remarkable paragraphs, which are quoted in translation, are therefore worthy of note, since they present not only the first, but to this day, the most complete description of the gross morphology of the new endocrine organs.

Lyonet, P., 1762. Traité Anatomique de la Chenille Qui Ronge le Bois de Saule. Pages 435–437.

The Granulated Vessels

"Before going on to examine the fat-body and the parts which it encases, there remains to be described two strange vessels, which, on account of their small size, we did not mention in the general outline which we have given in Chapter VI on the interior parts of the caterpillar.

"These vessels, which, on account of their structure, will be called the granulated vessels, are located on the tracheae on the posterior side of the prothoracic spiracle where they form a semi-circle around trachea *a*. They pass dorsally along the tracheal branches *b* and *c*, the muscle *d*, and the cephalic tracheae, *e* and *f*. Each ends on its respective side between the cephalic tracheae *f* and *g*.

"They were consistently present in all the caterpillars of this species which I examined. On account of their small size they are difficult to distinguish easily without a magnifying glass, and they may be mistaken at first for a section of fat. When viewed through the microscope, they appear as they are represented in Pl. XII Fig. 8; in other words, like a long, narrow, irregular and curved mass of longish adherent grains of varying sizes, usually smaller in the direction of the superior line and at the points of insertion of the nerves and tracheae.

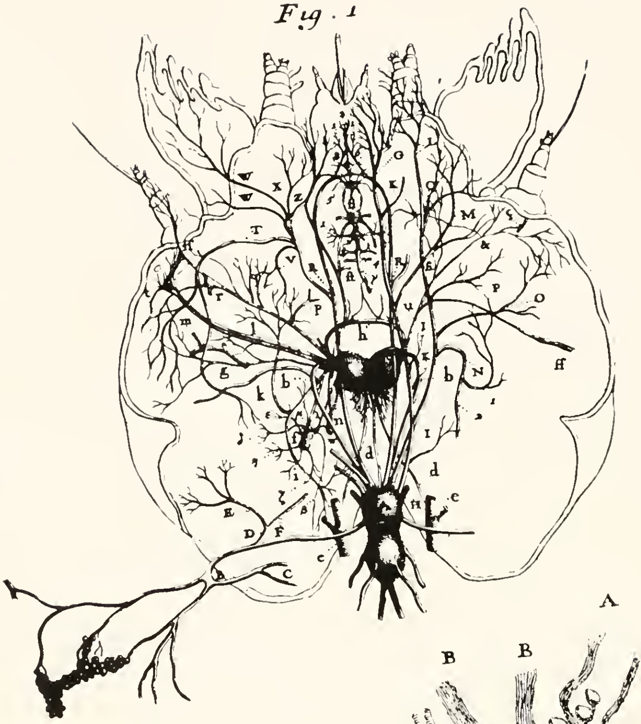
"When this mass of grains is dissected, it is found to be formed by a long membranous sac loaded with small blisters which open on it and which are filled, as is the sac, with white matter which presents nothing specific.

Nerves. "In the subject from which Fig. 8 was taken, the nerves A,A,A,A, which supply the granulated vessels seemed to me to come from the third and fourth branch and from the second subdivision of the second branch of the last pair of nerves from the second ganglion; those marked B,B, from the second pair of nerves from the third ganglion; and those marked C,C,C, from the first spinal connectives.

Tracheae. "The tracheae which insert here seemed to me to come from the first and from the second cephalic trunks, but I have neglected to examine, as should have been done, the tracheae and nerves of this small section.

Function. "I have not been able to discover the purpose of the granulated vessels. The relationship they have to the ovaries of some insects could cause them to be taken for real ovaries.

Fig. 1



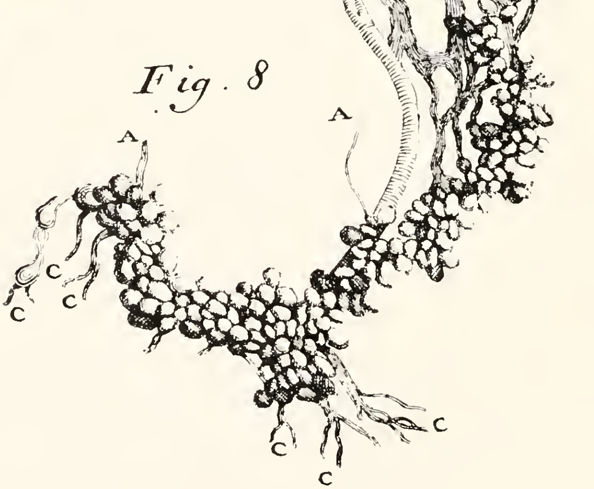


Fig. 8

FIGURE 1. From Lyonet's Plate XII. Dissection of the head of the caterpillar as seen in ventral view. Note the prothoracic gland attached to its nerves in the lower left hand corner of the figure.

FIGURE 8. From Lyonet's Plate XVIII. Enlarged view of a prothoracic gland, showing innervation and tracheal supply.

> But their location and form, which are very different from that of the ovary of the moth of this caterpillar, indicate the contrary. One might possibly suspect that they are the rudiments of the wings of this animal. This idea occurred to me first; but I was soon corrected when, upon opening a caterpillar on the point of changing into a pupa, I distinctly recognized the wings of the moth, and did not fail to find also the granulated vessels which had not changed shape. It is therefore only through studies of the anatomy of the pupa or the moth that one may hope to discover something on this point."

Nearly two centuries elapsed before application was made of the advice of the final paragraph. Then, in a series of investigations reported in 1940, 1941, and 1944, Fukuda was able to demonstrate that the prothoracic glands of *Bombyx mori* were involved in the endocrinological control of moulting, pupation, and adult development.

In the past few years certain of Fukuda's observations have been confirmed on other insects. In the Cecropia silkworm, for example, the development of the adult moth within the pupa requires a hormone secreted by the prothoracic glands (Williams, 1947, 1949a). But, in addition to this factor, the initiation of adult development also requires the *presence* of a second hormone secreted by the brain (Williams, 1946, 1947, 1949b) and the *absence* of a third hormone secreted by the corpora allata (Williams, 1949c).

Evidence of this type makes it increasingly clear that neither the prothoracic glands nor any other organ are endowed with the complete control over metamorphosis. In insects as in mammals, hormonal mechanisms seem to require a certain complexity in order to prove feasible and self-balancing.

From these considerations we are led to a somewhat more moderate evaluation of the role of the prothoracic glands than that which Fukuda has proposed. The prothoracic glands are but one component in an endocrinological system that presides over metamorphosis: among the other components in this system must be included at least two further organs, the brain and the corpora allata.

Notwithstanding this complication, the identification of the prothoracic glands of Lepidoptera as endocrine organs has provoked a re-examination of the endocrinology of metamorphosis and a search for comparable organs in other orders of insects.

The glands are now known to occur, not only in the Lepidoptera (Lee, 1948), but also in the Hymenoptera (Williams, 1948). Apparently homologous organs have been described as "prothoracic glands" in the Orthoptera (Scharrer, 1948), as "intersegmental organs" in the Odonata (Cazal, 1947, 1948; Deroux-Stralla, 1948a), and as "ventral glands" in certain other hemimetabolous insects (Pflugfelder, 1947). In the case of the Odonata the anatomical affinity between intersegmental organs and prothoracic glands has been reinforced by experimental evidence. Thus, according to Deroux-Stralla (1948b), the removal of the intersegmental organs results in abnormalities in moulting and suppression of adult development—effects that would be anticipated in terms of the proposed homology.

Since our knowledge of insect endocrinology is based so largely on studies of the Hemiptera, it is significant that organs anatomically identical with prothoracic glands have been found in nymphs of the lygaeid, *Oncopeltus fasciatus* (Edwards, 1948).

Of further interest is the description by Possompès (1946) of a pair of "peritracheal glands" in larvae of Chironomus. Possompès calls attention to the histological resemblance between these peritracheal glands in the lower Diptera and the lateral cells in the ring-gland of the higher Diptera. If the ring-gland should prove to contain a component homologous to the prothoracic glands of other insects, it would account for some of the peculiar endocrinological effects of this organ.

Thus, notwithstanding the fragmentary character of the present evidence, it seems probable that prothoracic glands and their homologues are widely distributed among insects. A more detailed evaluation of their role in development may lead to a sounder understanding of metamorphosis and may open to experimental attack certain facets of the problem that have hitherto remained inaccessible. In accordance with the advice of Lyonet, it is only through further study that we may hope to discover something on this point.

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THE EFFECTS OF ATMOSPHERIC PRESSURE AND COMPOSITION ON THE FLIGHT OF DROSOPHILA

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In the absence of a satisfactory theory of minute airfoils, not to mention airfoils which like the insect wing undergo continuous and variable angular motion, only limited help in the detailed analysis of insect flight can be obtained at present from the science of aerodynamics. Nevertheless, it is worthwhile to attempt to apply certain elementary aerodynamic concepts in an effort to develop a rational basis for the study of the flight process.

One such approach is to regard the wings of an insect as diminutive paddles which in each wingbeat serve to impart a specific average velocity to a specific mass of air. This point of view has already proved useful in analyzing the correlation between frequency of wingbeat and the dimensions of the wings and thoracic muscles in various species of Drosophila (Reed, Williams and Chadwick, 1942). Considering flight of an insect in these simple terms, the power output (P) should be proportional to the mass of air moved per beat (m), the square of the average velocity (v) imparted to this mass, and the wingbeat frequency (f):

$$P \propto mv^2f. \tag{1}$$

For present purposes the relationship may be further simplified, since

$$v \propto f$$

and, therefore, substituting in Equation (1),

$$P \propto mf^3,$$

or

$$f^3 \propto P/m. \tag{2}$$

Thus, according to this analysis, the cube of wingbeat frequency should be proportional to the power output divided by the mass of air moved per beat. That this relationship has real validity is indicated by correlations already demonstrated between wingbeat frequency and the energy consumption during flight. In the case of Drosophila the flight energy (oxygen consumption and carbon dioxide production) was found actually to vary as the cube of wingbeat frequency (Chadwick and Gilmour, 1940; Chadwick, 1947). Manifestly, such a correlation would be expected on the basis of Equation (2), provided that the mass of air moved in each remained constant.

While the basic relationship has then been verified experimentally, the effects of varying the mass of air moved per beat have in contrast remained very largely a matter of speculation. V. Buddenbrock (1919), Roch (1922) and Sotavalta (1947) adopted the simplest means of altering this factor by clipping the tips from the wings. Though these investigators have offered various interpretations of their results, the increase in wingbeat frequency they observed seems to us attributable directly to the decreased mass of air moved per beat. However, the quantitative aspects are not readily established in experiments of this sort, which we have also performed, for the reasons that surfaces of equal area from different parts of the wing may not be equivalent aerodynamically and that we have as yet no means of measuring their presumably different contributions to the outflowing air stream. Thus, while one may observe that progressively shortening the wings results in a progressive increase in the rate of wingbeat, one is unable to derive a precise statement of the relationship thereby revealed. Such experiments suffer also, of course, from the necessity of mutilating the structure one is attempting to study.

Fortunately these difficulties can be avoided relatively easily, since, if the wings are regarded as sweeping out a specific volume of air with each beat, the mass of air moved is obviously dependent on the gaseous density of the medium. By varying the density, the wings may be made to sweep out a volume whose mass may be altered continuously, and the resulting changes in performance correlated, in terms of wingbeat frequency, with the density change. Alterations of density are produced and measured conveniently merely by varying the pressure of the air in which the insect flies. Our problem resolves itself then into an examination of the effects of changes in atmospheric pressure on the rate of wingbeat.

As far as we are aware, there has been no adequate investigation of this matter. Magnan (1934) states that "frequency changes with pressure also. Thus a fly making 160 strokes per second makes 20 more when placed in a vacuum corresponding to an altitude of 2000 meters." By an acoustic method Sotavalta (1947) determined the wingbeat frequencies of several species of bees at a series of subatmospheric pressures, but observed no deviations from the rates measured under normal conditions. Case and Haldane (1941) note in passing that Drosophila, exposed to an air pressure of 10 atmospheres, was unable to fly. These limited observations are all we have been able to find in the literature.

Our initial and simple objective of studying the effects of variation in atmospheric pressure has, as is so frequently the case, raised more problems than were contemplated at the outset. For, in addition to altering density, variations in air pressure produce systematic changes in oxygen tension and in pressure as such. On this account, efforts to study each of these factors separately were necessary. Measurements of the oxygen consumption during flight at normal and reduced pressures were also made when it became apparent that, for an understanding of the other data, more information was needed about the energy relationships concerned.

MATERIALS AND METHODS

A. Measurements of wingbeat frequency

Our first experiments were performed on *Drosophila repleta* Wollaston, a species particularly adapted to these studies on account of its dependable tarsal flight reflex. Later, *D. virilis* Sturtevant was also used. In working with *D. repleta,* individuals were selected at random from a wild population that maintained itself in the animal rooms and were used without regard to age or sex, since we were unaware at the time that frequency of wingbeat is determined to some extent by these variables. This defect in technique has contributed to the scatter in the data, and was avoided in the studies with *D. virilis,* which were grown under standard conditions at 25 degrees C. and isolated daily on emergence.

The apparatus in which wingbeat frequency was measured is a simplified version of the flight chamber described by Williams and Chadwick (1943), and has been diagrammed in Figure 1. It consists of a glass pressure chamber whose temperature was controlled either by circulation of water through a surrounding jacket or by immersion in a constant temperature bath. Provisions were made for clamping rubber stoppers, one of which held a thermometer, into the ends of the pressure chamber, while glass tubes, passing through the stoppers, allowed gas mixtures of known composition to be circulated.

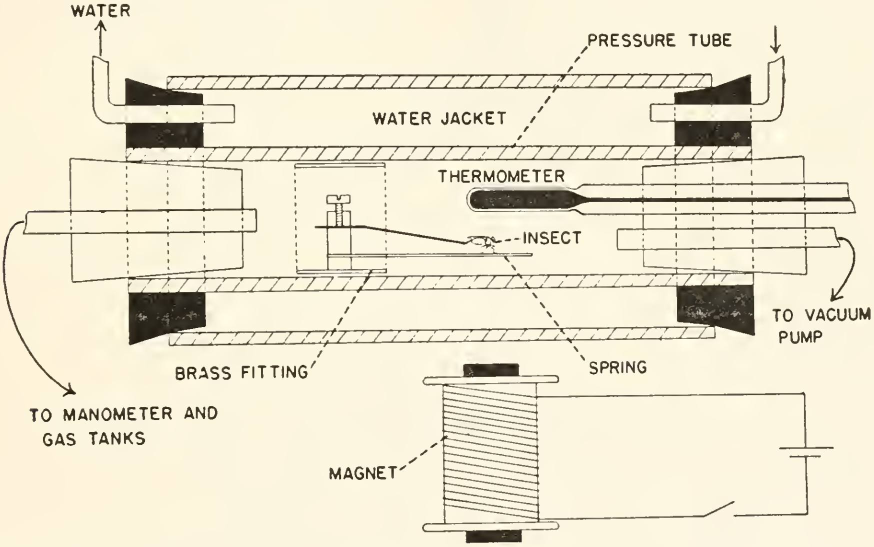

FIGURE 1. Apparatus for measurement of wingbeat frequency at various pressures. For explanation see text.

These mixtures were made up at high pressure in commercial gas cylinders and analyzed before use. At each change of gas mixture the chamber was washed thoroughly with the succeeding mixture. Pressure within the chamber was varied by the addition of compressed gases or by means of a vacuum pump. A pressure gauge and mercury manometer, measuring up to 5 atmospheres, were sealed into the gas lines and permitted a continuous check on the pressure within the experimental chamber. Relative humidity was held at or near 100 per cent by placing a few drops of water within the chamber and by bubbling the gases through water as they entered.

The rate at which pressures were altered between successive sets of measurements had no obvious effect on wingbeat frequency. Ordinarily these changes were made fairly quickly and though several minutes were then allowed for equilibration even this period of adaptation appeared unnecessary. It was possible therefore to test the various pressures in rapid random succession.

All the data obtained with *D. repleta* have been computed in terms of the response at 25 degrees C. In some of the earlier experiments the measurements were made at temperatures that deviated slightly from 25 degrees C.; these have been adjusted by applying a factor derived from studies of the effect of temperature on wingbeat frequency. Although the validity of this treatment was tested and confirmed, it became unnecessary in all later experiments when the flight chamber was maintained at 25 ± 0.1 degrees C. With *D. virilis,* various constant temperatures were used, as stated in the tabulation of results.

Measurements of wingbeat frequency were made on fastened specimens according to the method previously described. To evoke and terminate the flight of the insect within the sealed chamber, the tarsal reflex was utilized. Flight was induced by withdrawing a spring platform from under the animal's feet and stopped by interrupting the current to the electromagnet shown in Figure 1.

Frequency of wingbeat was measured by means of a General Radio "Strobotac." In each instance, the maximal frequency, occurring within the first few seconds of flight, was recorded. Each flight was therefore extremely brief, with a duration in most cases of about 2 seconds. In this way the onset of fatigue was postponed, so that several hundred measurements could be made on most individuals. Determinations were made at intervals of 10 seconds, the 8 seconds of rest between flights having been found adequate for recovery. The response of each individual to each experimental condition was usually recorded as the mean of 20 measurements.

Each fly was tested initially in air at atmospheric pressure and then under a variety of experimental conditions. At intervals during an experiment the performance was rechecked in air; any animal showing significant deviation from its initial response was discarded. Since it was impossible to test all individuals under all circumstances and since there were considerable differences in the wingbeat frequencies of different individuals in air at 760 mm. Hg (mainly because of our ignorance of the influence of age and sex), results for *D. repleta* have been calculated in terms of the deviation in frequency under each set of conditions from the frequency observed for that individual in air at 760 mm. Hg. For presentation of the average data as in Tables 1, 3, and 6, the average deviations in wingbeat frequency at each pressure have been added to or subtracted from the mean frequency for all individuals at 760 mm. Hg in air, in order to provide a more direct comparison of the rates in the various media used. Thus, for example, the average frequency of 10,700 cycles per minute at 3860 mm. Hg shown in Table 1 was obtained by subtracting 1990, the average decrement for these 17 animals from their rates at 760 mm., from 12,690, the mean for all 72 flies at normal pressure. The statistically preferable procedure of using a different randomly selected sample of flies for each set of conditions would have been impractical, particularly since we had not then succeeded in establishing *D. repleta* in culture.

With *D. virilis*, a series of only 4 or 5 pressures was used, and each insect was flown 10 times at each pressure. The results thus obtained were averaged and are presented in this form in Table 2.

B. Measurements of oxygen consumption during flight

Details of the technique for measuring oxygen consumption of Drosophila in flight have been described in previous reports (Chadwick and Gilmour, 1940; Chadwick, 1947). In the present study, differential volumeters (Fenn) were used and an arrangement adopted which allowed simultaneous evacuation of both vessels, as shown in Figure 2. The vessels had capacities of about 13 ml. and were connected by a capillary with a volume of about 5 cu.mm. per cm.

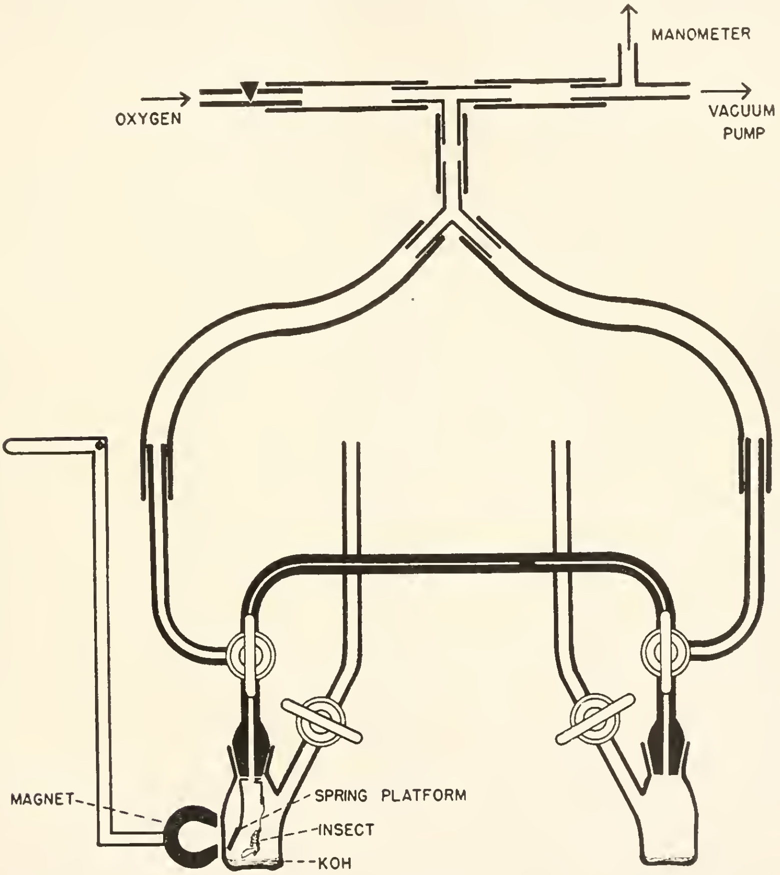

FIGURE 2. Apparatus for measurement of oxygen consumption during flight at normal and reduced pressures. For explanation see text.

Individual *D. virilis* of known age were anesthetized with carbon dioxide gas and fastened with paraffin to a fine wire which was then attached to the head of the respirometer. The fly was suspended head-down in the vessel, its feet in contact with the usual retractible platform. To depress the paraffin-coated platform and induce flight, a small permanent magnet was brought up to the side of the vessel in the bath. Carbon dioxide given off by the insect was absorbed in 0.1 ml.

of 15 per cent KOH in the bottom of the vessel, which had been fitted with a sleeve of filter paper to increase the absorbing surface.

During a 20-minute period of equilibration at 19.3 ± 0.01 degrees C. in the water bath, both vessels were gassed out with oxygen from a commercial cylinder. The stopcocks were then turned to running position and the resting oxygen consumption measured for 30 minutes or longer. In about half the experiments, flight was then induced at normal pressure and allowed to continue for 10 or 20 minutes, during which time readings of oxygen consumption were taken every minute and measurements of the rate of wingbeat every 10 seconds. For the latter, the specimen was viewed in silhouette against the flash lamp, which had been let down into the bath inside a glass cylinder. After the measurements at normal pressure, the system was evacuated to 200 or 400 mm. Hg, and the same procedure repeated. In the remaining experiments, the order of pressures was reversed; for example, the first set of measurements was made at 200 or 400 mm. Hg, and the second at normal pressure.

TABLE 1

Wingbeat frequency as a function of air pressure and density
Drosophila repleta in moist air at 25° C.

Air pressure mm. Hg	Density gms./liter	Average wingbeat frequency beats/min.	Number of specimens	Number of measurements
3860	6.00	10,700	17	322
3450	5.36	10,910	22	442
3100	4.82	10,990	25	525
2820	4.38	10,940	21	430
2580	4.01	11,330	20	425
2320	3.60	11,330	20	410
2100	3.26	11,660	21	425
1880	2.93	11,570	15	270
1660	2.57	11,720	13	224
1380	2.14	12,050	13	216
1200	1.86	12,110	14	250
980	1.51	12,320	15	310
760	1.17	12,690	72	2730
680	1.05	12,880	22	399
600	0.92	12,930	20	358
500	0.77	13,070	20	390
400	0.61	13,210	37	770
300	0.45	13,310	20	353
260	0.39	13,540	19	824
200	0.30	13,680	32	615
140	0.20	13,950	18	347
100	0.14	14,060	14	237

Careful attention to the lubrication and seating of stopcocks and other joints was essential since an inward leak amounting to a fraction of a cu.mm. per minute could render the measurements of oxygen consumption at low pressures valueless. Vaseline was used successfully as a stopcock grease at temperatures of 20 degrees C. or less, but although this and several other lubricants were tried, attempts to repeat these experiments at 26 degrees C. failed because leaks around the stopcocks invariably developed before a run could be completed. Only those experiments were considered valid in which a reasonably constant rate of resting oxygen consumption, of a reasonable magnitude in comparison with earlier measurements, was obtained for at least one half-hour before flight at each pressure, and in which the rate of oxygen consumption returned to and maintained a value approximating the preflight level within a few minutes after flight had ceased.

Although it was impossible in the system diagrammed to be certain that leakage was zero, one can state with assurance that any leaks which did occur were not greater than the average resting rate of about 30 cu.mm. per gm. per minute. Since the flight oxygen consumption was computed by subtracting the resting rate from the total measured during the flight which followed immediately, such errors would affect mainly the resting rates rather than the flight respiration in which we were chiefly interested. However, if one makes the highly conservative allowance of a possible error of 30 cu.mm. per gm. per minute, this will amount only to some 8–9 per cent of the average flight oxygen consumption measured at 19.3 degrees C. and will not alter significantly the conclusions we have drawn from the data.

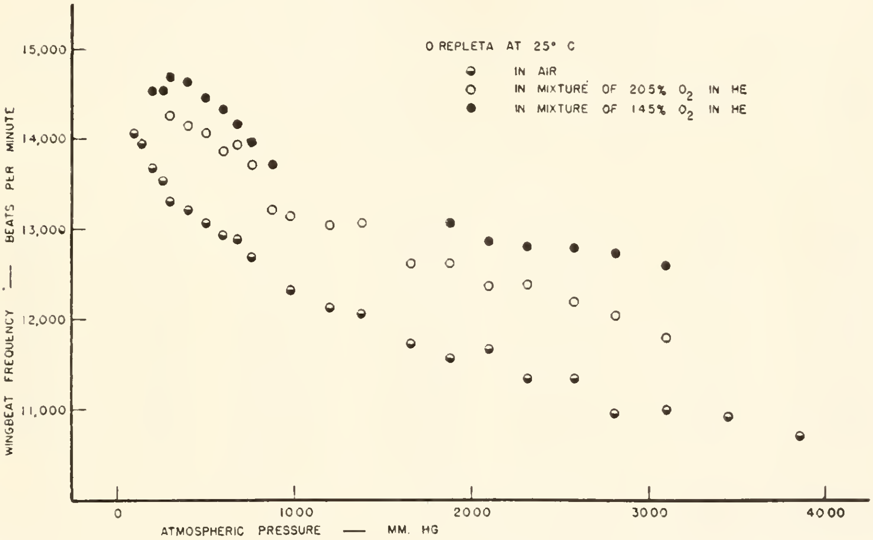

FIGURE 3. Wingbeat frequency of *D. repleta* as a function of atmospheric pressure in air and in two oxygen-helium mixtures.

Difficulty was experienced in some of the early attempts in obtaining a flight response from the insect after the vessels had been lowered into the bath. Illuminating the experimental vessel with a 40-watt bulb in a reflector at the side of the bath overcame this trouble. The lamp neither interfered with the observations of wing movement nor, since the glass wall of the bath and at least 6 inches of well-stirred water separated it from the respirometer, disturbed the measurements of oxygen consumption. In blank runs, no movement of the index drop occurred as a result of turning the lamp or stroboscope on or off.

RESULTS

A. Observations of wingbeat frequency

In order to have a means of differentiating between effects due to variation in total pressure, oxygen tension and gaseous density, *D. repleta* was tested in 5 different media: (1) air, (2) 14.7 per cent oxygen in nitrogen, (3) nitrogen-oxygen mixtures of higher oxygen content than air, (4) 14.5 per cent oxygen in helium and (5) 20.5 per cent oxygen in helium. The rate of wingbeat of *D. virilis* was measured in air at 5 different pressures, both at 19.3 degrees C. and at 25.9 degrees C.; and at normal pressure, 25.9 degrees C., in a mixture of 6.1 per cent oxygen in nitrogen.

TABLE 2

Wingbeat frequency of D. virilis as a function of air pressure

Pressure in mm. Hg.		1520	760	400	200	100
Specimen number	Age in days	Wingbeat frequency in beats per minute				
a. Moist air at 19.3 degrees C.						
3–112 ♀	8–9	10,690	11,000	11,680	12,660	13,180
3–113 ♀	8–9	8,740	9,550	10,390	11,280	refused
3–151 ♀	12–13	11,120	11,470	12,060	12,980	refused
3–152 ♀	12–13	10,410	11,100	11,930	12,770	refused
3–153 ♀	12–13	10,100	11,150	11,650	12,220	refused
3–155 ♀	12–13	10,570	11,290	12,300	13,390	14,020
3–157 ♀	12–13	10,630	10,870	11,620	12,560	refused
3–158 ♀	12–13	9,210	10,380	10,630	11,500	refused
3–159 ♀	12–13	10,950	11,120	11,720	12,030	13,060
3–1512 ♀	12–13	11,030	11,390	11,960	12,750	13,380
Average (10 flies)		10,350	10,930	11,590	12,410	—
Average (4 flies which flew at 100 mm.)		10,810	11,200	11,920	12,710	13,410
b. Moist air at 25.9 degrees C.						
3–211 ♀	4–5	13,710	14,010	14,870	15,670	16,370
3–212 ♀	4–5	13,870	14,290	14,770	15,590	16,130
3–213 ♀	4–5	14,250	14,510	14,810	15,670	15,790
3–214 ♀	4–5	13,650	13,990	14,290	14,650	15,150
3–215 ♀	4–5	13,450	14,050	14,710	15,550	15,970
3–217 ♀	4–5	14,170	14,590	15,290	16,010	16,250
3–218 ♀	4–5	13,790	14,210	14,930	15,670	15,850
3–221 ♀	5–6	12,050	12,880	14,090	15,510	refused
3–222 ♀	5–6	12,970	13,590	14,530	15,630	16,050
3–223 ♀	5–6	10,320	10,950	12,330	13,370	13,940
Average (10 flies)		13,250	13,710	14,460	15,330	—
Average (9 flies which flew at 100 mm.)		13,350	13,800	14,500	15,310	15,720

Each observation is the mean of 10 measurements.

1. *Air*

The effects of variation in air pressure on the frequency of wingbeat of *D. repleta* were studied in a total of 72 individuals, over a range of 5 atmospheres. As indicated in Table 1 and Figure 3, the frequency of wingbeat decreased gradually as the pressure increased. This effect was observed over the whole range of pressures investigated, from 80–100 mm. Hg, below which the animals failed to fly when stimulated, to a pressure of 3860 mm. Hg.

Examination of the data reveals an apparent discontinuity in the relationship at about 680 mm. Hg. This is best visualized on the logarithmic grid of Figure 4. Our reasons for considering it an artefact are given in the discussion. No such discontinuity is evident in the data obtained at two temperatures with *D. virilis* (Table 2 and Figure 4).

TABLE 3

Wingbeat frequency as a function of pressure in a mixture of 14.7 per cent oxygen in nitrogen
Drosophila repleta at 25° C.

Total pressure mm. Hg	Density gms./liter	Average wingbeat frequency beats/min.	Number of specimens	Number of measurements
3100	4.78	10,950	12	202
2820	4.34	11,090	7	140
2320	3.57	11,480	8	87
1880	2.89	11,610	12	190
1380	2.12	11,960	18	290
980	1.50	12,220	19	370
760	1.16	12,600	39	1197
680	1.04	12,860	12	170
600	0.91	12,890	5	70
500	0.76	13,300	11	157
400	0.60	13,260	9	140
300	0.45	13,540	10	146
200	0.30	13,970	4	55

2. *14.7 per cent oxygen in nitrogen*

A further series of 39 *D. repleta* was tested in a mixture of subnormal oxygen content in order to emphasize the possible effects of decreased oxygen tension at low total pressures. As shown in Table 3 and Figure 4, no significant difference was evident in comparison with the relationship observed in air. Comparative data for *D. virilis* in air and in 6.1 per cent oxygen in nitrogen are given in Table 4. Again no difference was observed.

3. *Atmospheres of high oxygen content*

By somewhat different measures the effects of subatmospheric pressures of mixtures with high oxygen content were studied. In these experiments each individual was tested at a specific low pressure in air and then subjected to the same pressure in an atmosphere rich in oxygen. The two sets of measurements, samples of which are given in Table 5, showed no significant differences. It was never possible to cause the frequency of wingbeat to rise above the value in air by supplying a greater than normal proportion of oxygen.

4. Helium-oxygen mixtures

Having found no correlation between the tension of oxygen and the response of the insect to pressure, there remained the problem of distinguishing between the two other variables involved in these experiments; namely, gaseous density and pressure *per se.* Their separation seemed difficult at first, since the density and total pressure of a given gas mixture are directly proportional. However, the fact that helium is an inert gas with a density only about one-seventh that of nitrogen offered a means of attacking the problem. Using helium and oxygen, mixtures may be prepared which differ from air or other oxygen-nitrogen mixtures in density but not, presumably, in regard to most other properties which are physiologically significant.

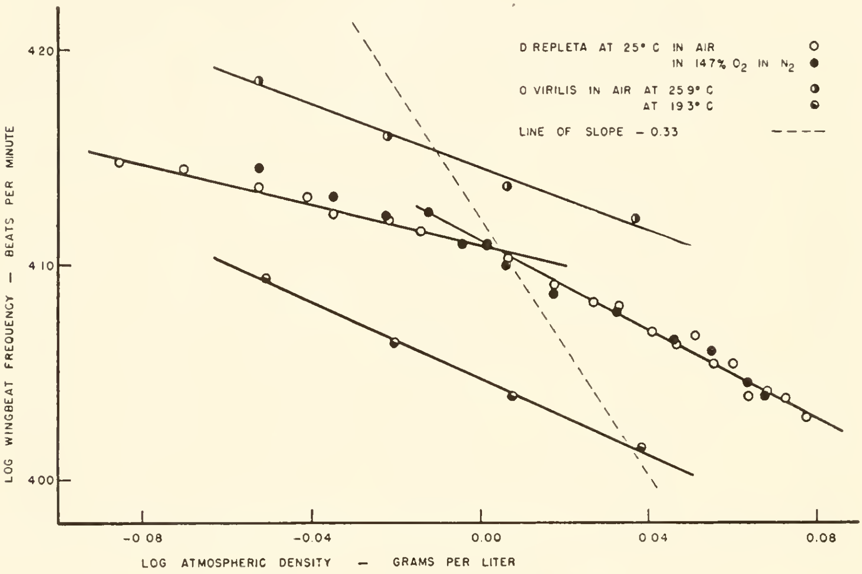

FIGURE 4. Wingbeat frequency of *D. repleta* and *D. virilis* as a function of atmospheric density. Solid lines fitted to empirical data by the method of least squares. Broken line of slope —0.33 added for comparison.

Two helium-oxygen mixtures were used for this purpose, one containing 14.5 per cent oxygen, the other 20.5 per cent. At 25 degrees C. and one atmosphere, the densities of these mixtures, both of which contained water vapor and small amounts of nitrogen, were approximately 0.33 and 0.39 grams per liter, respectively, as compared with 1.17 grams per liter, the density of moist air.

Frequency of wingbeat was measured with *D. repleta* in each of these mixtures throughout most of the range of pressures with results recorded in Table 6 and Figure 3. It is evident that the frequency was higher in either mixture than in

TABLE 4

Wingbeat frequency of D. virilis as a function of oxygen tension
Moist gas at 25.9 degrees C. and 760 mm. Hg

Specimen number	Age days	Wingbeat frequency in Air	6.1 per cent O_2 in N_2
		beats per minute	
3-214 ♀	4-5	13,240	13,220
3-215 ♀	4-5	13,300	13,180
3-217 ♀	4-5	13,840	13,940
3-218 ♀	4-5	13,460	13,120
3-221 ♀	5-6	12,900	12,900
3-222 ♀	5-6	13,700	13,820
3-223 ♀	5-6	10,740	10,690*
3-232 ♀	6-7	12,670	12,710
3-234 ♀	6-7	11,730	11,950
3-235 ♀	6-7	13,140	13,210
Average (10 flies)		12,870	12,870

* Rate of this specimen apparently depressed by previous flights; initially the rate was 10,950 in air at 760 mm. Hg (Table 2).

Each observation is the mean of 10 measurements.

air of the same pressure, and that it was highest at any given total pressure in the mixture having the least density.

During these experiments the animals reacted badly to the helium mixtures. It was frequently difficult or impossible to induce flight with the usual stimulus, and, after a relatively small number of flights, the wingbeat frequency began to decrease. The behavior resembled that of a fatigued animal in air, and on this account it was

TABLE 5

Wingbeat frequency in air and in atmospheres with greater oxygen content
Drosophila repleta at 25° C.

Specimen number	Pressure mm. Hg	Wingbeat frequency in Air beats/min.	O_2-N_2 Mixture beats/min.	Oxygen content of mixture per cent
97	1660	13,060	12,590	100
87	680	14,310	14,000	100
93	600	12,060*	11,980	100
90	400	13,430	13,390	44
97	200	14,440	14,440	100
67	200	15,080	14,370	50
64	100	16,380	15,870	50

* In 3.9 per cent oxygen in nitrogen.

Each frequency datum is the mean of 20 observations. Atmospheres were saturated with water vapor at 25° C.

necessary to reduce the number of flights. Thus the points determined for individual specimens during this series of experiments have been averaged as a rule from only five measurements at each pressure. Specimens which had become refractory in the helium mixtures resumed a normal behavior when returned to air. It was observed also that the animals responded more readily in the helium mixtures when the total pressure was high than when it was one atmosphere or less.

TABLE 6

Wingbeat frequency as a function of pressure and density in helium-oxygen mixtures
Drosophila repleta at 25° C.

Total pressure mm. Hg	Density gms./liter	Average wingbeat frequency beats/min.	Number of specimens	Number of measurements
a. 14.5 per cent oxygen in helium				
3100	1.34	12,590	10	50
2820	1.22	12,730	9	45
2580	1.12	12,780	9	45
2320	1.00	12,800	8	40
2100	0.91	12,860	8	40
1880	0.81	13,070	8	40
880	0.38	13,720	9	45
760	0.33	13,960	15	75
680	0.29	14,160	5	25
600	0.26	14,330	5	25
500	0.21	14,460	7	35
400	0.17	14,640	7	35
300	0.13	14,680	6	30
260	0.11	14,530	5	25
200	0.08	14,530	5	25
b. 20.5 per cent oxygen in helium				
3100	1.62	11,780	7	50
2820	1.47	12,030	7	50
2580	1.35	12,190	8	55
2320	1.21	12,390	7	40
2100	1.10	12,370	7	35
1880	0.98	12,610	8	40
1660	0.87	12,610	7	45
1380	0.72	13,070	8	35
1200	0.62	13,040	7	35
980	0.51	13,140	9	45
880	0.46	13,210	8	40
760	0.39	13,710	22	135
680	0.35	13,930	8	40
600	0.31	13,870	10	50
500	0.26	14,060	11	55
400	0.21	14,150	9	45
300	0.15	14,260	7	35

5. *Other observations*

a. Humidity. In an early series of experiments we found that the frequency of wingbeat failed to increase at subatmospheric pressures when the relative humidity within the flight chamber was low. On the contrary, the rate decreased rapidly and the specimens soon became incapacitated. This effect seems explicable in terms of damage to the insect from loss of water; possibly this factor may account for the negative results reported by Sotavalta (1947).

b. Stroke amplitude. During measurements at high pressures a reduction in the stroke amplitude was evident in most individuals. Though the magnitude of these changes was not measured, they are of considerable importance theoretically, as will be brought out in the discussion.

Table 7

Wingbeat frequency of D. repleta before and after removal of halteres

Specimen number	Wingbeat frequency in beats per minute after treatment indicated		
	Etherized and mounted	Re-etherized	Again re-etherized and halteres removed
48 ♀	10,220	10,190	10,250
49 ♂	9,640	9,800	9,930
50 ♂	9,950	10,130	9,890
54 ♀	10,660	10,670	10,490
55 ♀	10,340	10,340	10,380
56 ♀	12,290	12,240	12,280
57 ♀	12,360	12,350	12,120
58 ♀	10,880	10,930	10,610
59 ♀	11,510	11,600	11,720
60 ♀	11,850	11,940	11,880
61 ♀	10,520	10,540	10,380
62 ♀	11,110	11,090	11,580
64 ♂	10,160	10,260	10,320
65 ♀	11,250	11,220	11,450
66 ♀	10,360	10,530	10,520
67 ♀	10,320	10,340	10,640
68 ♀	11,150	11,120	11,210
Average	10,850	10,900	10,920
Standard error	±194	±180	±188

Each datum is the average of 20 determinations. The experiments were run in moist air at 20° C. and 615 mm. Hg.

c. Halteres. An important rôle of the halteres in regulating the wingstroke has been proposed frequently in the past and reemphasized recently by Pringle (1948), so that it seemed advisable to give some attention to these organs under the conditions of our experiments. As originally reported by Williams and Reed (1944) and subsequently confirmed by Pringle (1948), the halteres are vibrated during flight at the same frequency as the wings, but in opposite phase. See also Curran (1948). This synchrony persists at all pressures. Furthermore, wingbeat frequency in air at various pressures was unaffected by amputation of the halteres as illustrated by the example given in Table 7. Even when the rate had been increased by clipping the wings, amputation of the halteres had no further effect (Table 8). The relationship between pressure and wingbeat frequency was apparently identical in the case of normal and haltereless flies; in fact, the only difference we have seen in the wing action of the two groups is in the somewhat steadier wingbeat frequency of haltereless flies during continuous flight.

d. Failure to respond at low pressures. In experiments at reduced pressure it was never possible to evoke flight when the total pressure was less than 80 to 100 mm. Hg. Of this pressure approximately 24 mm. Hg must be assigned to water vapor so that, in air, the partial pressure of oxygen amounted only to some 12 to 16 mm. Hg. Under such circumstances one might suppose that failure to fly was due to oxygen lack. Yet flight at lower pressures was still unobtainable when pure oxygen was substituted for air. This rather surprising observation was verified repeatedly.

TABLE 8

Wingbeat frequency of D. repleta before and after clipping wings and removing halteres

Specimen number	Wingbeat frequency in beats per minute after treatment indicated				
	Etherized and mounted	One wing clipped	Both wings clipped	Re-etherized	Again re-etherized and halteres removed
34 ♂	12,230	12,980	14,170	14,050	14,010
37 ♀	12,680	13,520	14,930	15,440	15,150
38 ♀	11,650	12,200	12,560	12,680	12,910
39 ♀	11,880	12,880	13,360	13,260	13,460
40 ♀	11,460	12,590	12,810	12,900	13,310
41 ♂	11,340	12,120	12,730	12,710	12,810
43 ♂	11,600	11,800	12,330	12,400	12,150
Average	11,850	12,580	13,270	13,750	13,400

Each datum is the average of 10 determinations. The experiments were run in moist air at 20° C. and 615 mm. Hg, except for those with Specimen Number 34, which were run at 22° C. and 645 mm. Hg. The observations are in contradiction with the finding of Roch (1922) that clipping one wing leaves the wingbeat frequency unaltered. Particular care was taken to cut the same amount from each wing, as nearly as possible; in general, from one-quarter to one-half the wing was removed, by a transverse cut.

e. Limiting tension of oxygen. Although some factor other than oxygen lack appears to prevent a flight response at total pressures below about 80 to 100 mm. Hg, there is also a lower limit to the oxygen tension consistent with brief interrupted flight. This is usually encountered when the oxygen tension in the gas mixtures is reduced below 15 to 20 mm. Hg. Thus in the case of air the limitation due to lowering the partial pressure of oxygen is about the same as that imposed by the unknown factor noted in the previous paragraph.

B. Measurements of oxygen consumption

Respiratory rates averaged over 10 or 20 minutes of continuous flight at normal and reduced pressure, together with the average rates of wingbeat observed simul-

TABLE 9

Oxygen consumption and wingbeat frequency of D. virilis during continuous flight at normal and reduced pressures in oxygen

Specimen number	Age	Weight	At 400 mm. Hg		At 760 mm. Hg		Ratio $\frac{O_2 \text{ at } 400 \text{ mm. Hg}}{O_2 \text{ at } 760 \text{ mm. Hg}}$
			Frequency	Oxygen consumption	Frequency	Oxygen consumption	
	days	mg.	beats per minute	cu. mm. per gm. per minute	beats per minute	cu. mm. per gm. per minute	
*2–181 ♀	8–9	2.51	10,760	341	10,340	310	1.10
*2–182 ♀	8–9	2.72	11,060	337	10,460	306	1.10
*2–211 ♀	12–13	2.22	11,020	404	10,710	391	1.03
*2–221 ♀	6–7	2.39	10,770	401	10,360	406	0.99
2–231 ♀	7–8	2.69	10,210	337	9,580	312	1.08
2–251 ♀	9–10	1.93	11,050	248	9,520	236	1.05
2–252 ♀	9–10	2.79	10,160	330	9,410	330	1.00
2–281 ♂	4–5	1.64	10,400	363	9,140	356	1.02
Average		2.36	10,680	345	9,940	331	1.05
			At 200 mm. Hg		At 760 mm. Hg		Ratio $\frac{O_2 \text{ at } 200 \text{ mm. Hg}}{O_2 \text{ at } 760 \text{ mm. Hg}}$
3–11 ♀	5–6	2.17	12,220	263	10,450	315	0.83
3–12 ♂	5–6	1.60	12,480	467	11,270	473	0.99
3–21 ♀	6–7	1.63	12,740	348	10,120	266	1.31
3–71 ♂	3–4	1.64	11,770	377	10,080	441	0.85
*3–72 ♀	3–4	1.90	12,050	345	10,200	409	0.84
*3–81 ♀	5–6	1.93	12,040	289	10,340	333	0.87
*3–91 ♀	6–7	2.25	10,910	310	9,670	320	0.97
*3–92 ♀	6–7	2.74	11,670	343	10,480	358	0.96
*3–101 ♀	7–8	1.84	11,210	308	10,270	294	1.05
Average		1.97	11,900	339	10,320	356	0.96

* Specimens thus marked were flown first at normal pressure; the others were flown first at reduced pressure.

Volumes corrected to NTP.

taneously, are presented in Table 9. Since the purpose of these measurements was to learn what effect alterations in density might have on the output of power by the flying insect, the runs were made in an oxygen atmosphere. As indicated above, the initial frequency of wingbeat appears to be independent of oxygen tension, but this is not true of the frequencies observed during continuous flight. In atmospheres with a low partial pressure of oxygen, the rate of wingbeat decreases rapidly after the first few seconds, and flight is maintained for a shorter period than under normal conditions. The rate of oxygen consumption also is depressed (Chadwick and Gilmour, 1940; Davis and Fraenkel, 1940). In the present experiments with *D. virilis* the tension of oxygen was higher than that of moist air at normal pressure even in the runs at a total pressure of only 200 mm. Hg, so that the results may be considered merely in reference to density change.

The flights were held to relatively short durations in order to minimize complications due to progressive fatigue, which reduces the rate at which oxygen is consumed. This factor was further equalized in the averages by reversing the order of pressures used in half the cases. The results thus obtained at 200 mm. Hg and 400 mm. Hg did not show any significant change in the average rate of oxygen consumption during flight at these pressures in comparison with the performance of the same individuals at 760 mm. Hg, although the data at 200 mm. Hg indicate a possibly significant depression for 4 of the 9 flies tested.

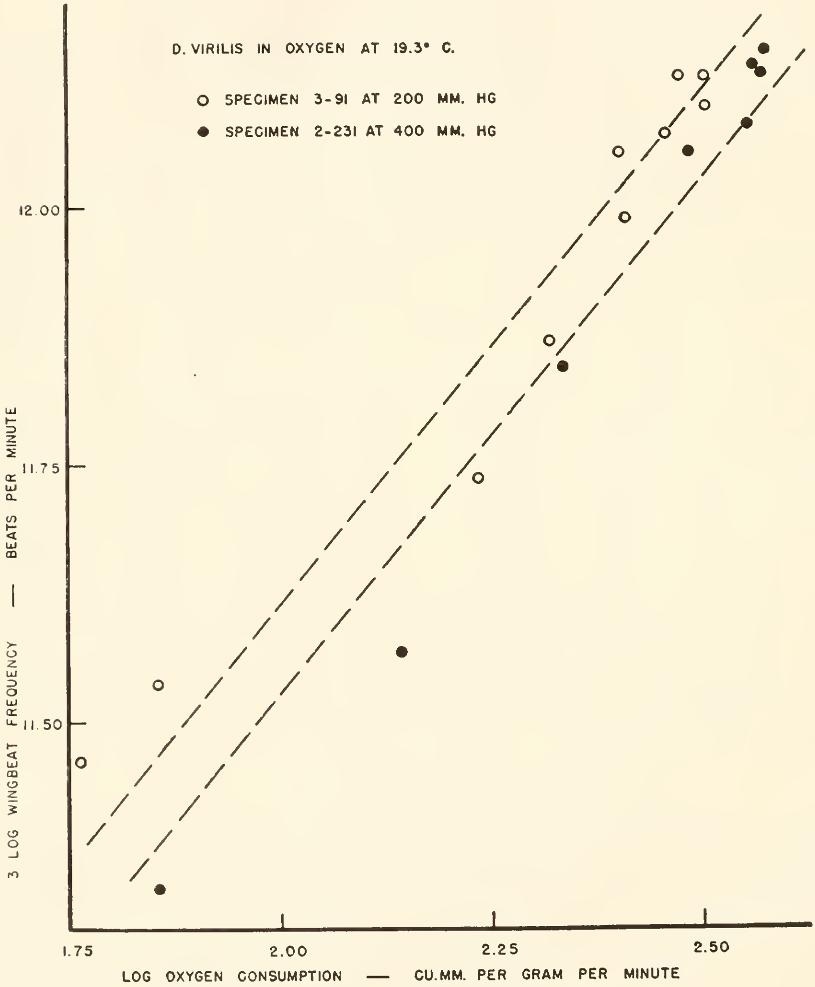

FIGURE 5. Correlation between wingbeat frequency and rate of oxygen consumption during flight in an oxygen atmosphere at reduced pressures. The broken lines have been drawn to conform to the equation, $3 \log F = \log k + \log O_2$, by using for $\log k$ the average value obtained for each specimen when the paired empirical values of wingbeat frequency and oxygen consumption were substituted into the above relationship. Each point represents the average values observed during 5 minutes of continuous flight.

The average oxygen uptake for 22 specimens, including some for which satisfactory runs were not obtained at reduced pressure, was 357 cu.mm. per gm. per minute at 19.3 degrees C. and 760 mm. Hg. These data agree closely with rates reported previously for flights of comparable duration with this species in air at 20 degrees C. (Chadwick, 1947). Thus it is apparent that the rate of oxygen consumption was not increased by supplying oxygen in excess of the tension normally present at one atmosphere.

In earlier studies a proportionality between the cube of wingbeat frequency and the rate at which oxygen is consumed (or CO_2 produced) was demonstrated for flights in air at normal pressure. Here, a few additional flights of about 40 minutes duration were made at 200 and 400 mm. Hg, and from them it was ascertained that the relationship

$$3 \log f = \log K + \log O_2 \qquad (3)$$

applies at pressures other than normal. Typical results of such runs have been plotted in Figure 5.

Discussion

The measurements reported above demonstrate that the principal factor concerned in the relationship between wingbeat frequency and atmospheric pressure is variation in gaseous density. A comparison of the values obtained in the helium mixtures with those obtained in air shows this clearly. In Figure 6 the three sets of data have been plotted together on coordinates where density, rather than total pressure, is the independent variable. Considering that the helium mixtures had

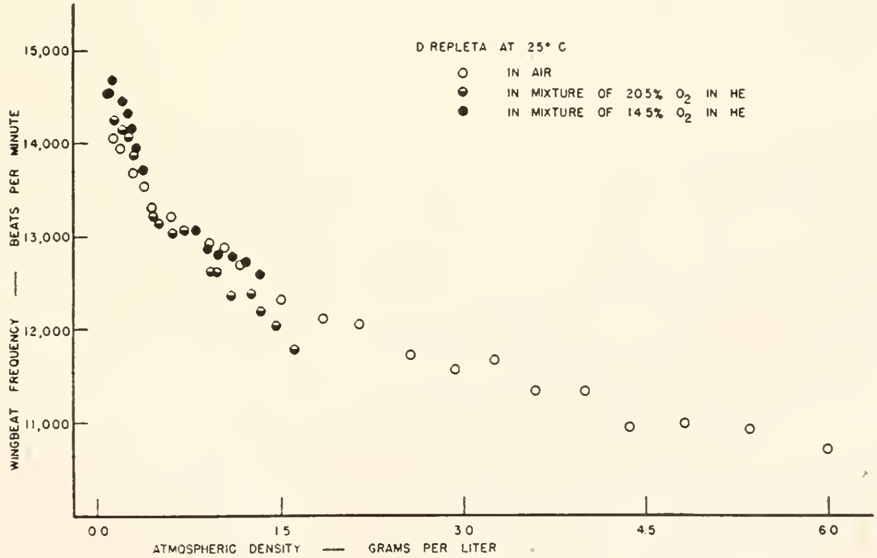

FIGURE 6. Wingbeat frequency of *D. repleta* as a function of atmospheric density in air and in two oxygen-helium mixtures.

detrimental effects upon the animals, and that there are in the curves irregularities due to various factors other than density, the agreement is convincing. Thus, at densities of about 1 gm. per liter, the average wingbeat frequencies in all the gas mixtures were identical within 2 per cent, although the determinations were made at less than one atmosphere in air, approximately 2½ atmospheres in the 20.5 per cent oxygen in helium mixture, and at over 3 atmospheres in the 14.5 per cent oxygen in helium mixture. The failure of variations in oxygen tension to exert any effect, so long as the partial pressure remained above the limiting value of 15–20 mm. Hg, is also visible in these series, as it is in a comparison of results obtained in air and other oxygen-nitrogen mixtures. It is evident, therefore, that the correlation between wingbeat frequency and pressure depends, in fact, upon a relationship between wingbeat frequency and gas density.

A noteworthy feature of this relationship is the relatively small magnitude of the observed effects. Even when the density of air was increased five-fold by compression, the frequency of wingbeat decreased only 16 per cent. That this effect is indeed a minor one may be judged from a comparison of the effectiveness of change in gas density with that of change in environmental temperature. The decrement that 5 atmospheres of pressure produced in wingbeat frequency can, for example, be duplicated by lowering environmental temperature only 5 or 6 degrees C.

Such small effects of variation in gas density would not be anticipated on the basis of Equation (2):

$$f^3 \propto P/m. \qquad (2)$$

The mass of air moved per beat is, obviously, equal to the product of stroke volume (V_s) and gas density (ρ). Hence

$$f^3 \propto P/V_s\rho. \qquad (4)$$

If the power output (P) and the stroke volume (V_s) remain constant, then frequency (f) should vary inversely as the cube root of gas density; that is, as $\rho^{-0.33}$.

The large deviation of the actual relationship from this theoretical one is amply evident in Figure 4. Equations fitted by the method of least squares to the data obtained with *D. repleta* in air at 25 degrees C. are

for densities below 1.05 gm./L.,

$$\log f = 4.1271 - 0.0460 \ (\log \rho + 0.3529); \qquad (5)$$

for densities above 1.05 gm./L.,

$$\log f = 4.0659 - 0.1028 \ (\log \rho - 0.4463). \qquad (6)$$

With *D. virilis,* tested at two temperatures over the pressure range from 2 atmospheres to 200 mm. Hg, the relationships are:

at 19.3 degrees C.,

$$\log f = 4.0530 - 0.0885 \ (\log \rho + 0.0623); \qquad (7)$$

at 25.9 degrees C.,

$$\log f = 4.1513 - 0.0726 \ (\log \rho + 0.0748); \qquad (8)$$

and there is no evidence of any discontinuity.

In the latter respect, the true picture, we believe, is that presented by the studies with *D. virilis.* Here the samples of flies were more homogeneous, and all specimens were flown at all of the pressures included in the averages. Even so, differences were noted in the slopes of the curves given by different individuals. With *D. repleta,* where flies were selected at random from a mixed wild population, it happened by chance that more individuals whose performance yielded rate-density curves with small slopes were tested at the lower pressures, and more individuals giving greater slopes at higher pressures. Averaging these groups together has produced a composite curve with somewhat different slopes in the positive and negative pressure ranges.

With both species, the slopes for individual animals range from about -0.03 to -0.15. These differences, which occur even in stocks reared under standard conditions, do not seem to be related to age or sex, and are not understood. It is unlikely that they depend on the factors involved in the correlation demonstrated by Reed et al. (1942), who showed that the rate of wingbeat is influenced by variations in the bodily dimensions which affect the ratio between muscle volume and the area of the wings. That this should be so is evident from Equation (4) above. Power output (P) will be proportional to the product of muscle cross-section and length; stroke volume (V_s), to the wing area. Hence, for a given air density, the wingbeat frequency will be less when the ratio P/V_s is small; i.e., when the wings are large relative to the power of the muscles which move them. But variation in the slope of the rate-density relationship cannot be ascribed to differences of this sort, for alterations in the ratio, P/V_s, should yield a family of parallel curves when the logarithm of wingbeat frequency is plotted against the logarithm of density.

Accepting these differences in slope as an unexplained phenomenon, we see nevertheless that whereas theory predicts the variation of f as $\rho^{-0.33}$, the actual measurements show f varying at a rate no greater than the -0.15 power of density. Since the theoretical relationship is based upon assumed constancy of stroke volume and power output, it is clear that one or both of these assumptions must break down when frequency changes in response to alterations in atmospheric density. Each of them must therefore be subjected to further examination.

In the absence of means for direct measurement of the power output of Drosophila at densities other than normal, we have turned to the rate of oxygen consumption as an index of this factor. The oxygen uptake gives a measure of the rate at which chemical energy is liberated by the active muscles and this figure, the power input (P_i), is related to P, the power output, through a factor, e, which represents the overall efficiency of the flight process:

$$P = eP_i. \tag{9}$$

Measurements reported above (Table 9) show that P_i is essentially independent of variation in density over the range from 200 mm. Hg to 760 mm. Hg, or suffers at most a slight decrease at the lower pressure. Apparently, the rate at which the muscles are able to liberate energy is limited largely by temperature and the physiological state of the insect in respect to fatigue, so that we may with reasonable safety extrapolate our findings at reduced pressures to cover the range of positive pressures in which measurements of oxygen consumption were not feasible with our apparatus, especially since we know that substitution of oxygen for air at normal pressure is without effect on the rate of oxygen consumption during flight. If this reasoning is accepted, we may then conclude that the power output also should be independent of density provided that varying the latter does not cause changes in efficiency.

In attempting to decide this last question, we are again hampered by lack of data, for to settle the problem would require measurements of both power output and power input at several positive and negative pressures. One might perhaps anticipate some decrement in the efficiency of the wings at reduced pressures. Should this occur, it would help to account for the failure of wingbeat frequency to rise as rapidly as predicted by Equation (4) when density is decreased; but it seems very unlikely that a several-fold increase in efficiency occurs at a pressure of 5 atmospheres, as the logical extension of this argument to the range of positive pressures would demand. Since the relationship between wingbeat frequency and density for the individual insect is continuous, without change in exponent over the entire range tested, it follows that any compensatory alteration which would account for the divergence from a line of slope -0.33 must also be continuous. We are thus led to infer that changes in efficiency must be relatively unimportant when wingbeat frequency is altered as a function of density.

By elimination, then, we are persuaded to look upon changes in stroke volume as the most probable source of the compensation needed, and we must now inquire whether differences of the required magnitude are reasonably likely. Taking an average value of -0.10 for the slope of the empirical rate-density relationship, we may set

$$f^3 \propto \rho^{-0.30}, \tag{10}$$

and on substitution of this value into Equation (4) we obtain, with constant power output,

$$V_s \propto \rho^{-0.70}. \tag{11}$$

On this basis, if stroke volume at normal density is taken as 1, values of about 4.4 and 0.3 would be required at 100 mm. Hg and 3860 mm. Hg respectively.

Our judgment as to whether alterations of this size are within reasonable limits will be assisted by the following analysis. Consider that the volume, V_s, swept out by the wings in each cycle is approximately the segment of a cylinder. The radius of this cylinder is the wing length, L; its height, h, is equivalent to the product of the average wing width, W, and the sine of the angle of attack, α:

$$h = W \sin \propto . \tag{12}$$

The angle of attack is defined as the angle between the chord of the wing and the relative wind; thus the effective height of our hypothetical cylinder is the projection of the mean width of the wing on a plane perpendicular to its direction of motion relative to the oncoming column of air.

Now the volume of the segment swept out by the wings in a complete cycle, including both up and downstroke, will be related to the volume of a cylinder with the above dimensions as twice the stroke amplitude is to 360 degrees. Remembering that there are two wings, we may then summarize as follows:

$$V_s = 2\pi \times L^2 \times W \sin \alpha \times (2 \times \text{amplitude}/360°), \tag{13}$$

or, since the wing dimensions are constant for a given specimen,

$$V_s \propto \text{amplitude} \times \sin \alpha. \tag{14}$$

Thus we see that the principal variables involved in the stroke volume are the stroke amplitude and the angle of attack.

From head-on photographs of *D. repleta* in flight the amplitude at normal pressure in a plane transverse to the body axis has been measured as approximately 135 degrees with the wing tips making contact at the extreme of the upstroke. While this arc could theoretically be cut to 45 degrees to account for the required decrease in stroke volume at a pressure of 5 atmospheres, although the decrement in amplitude observed visually does not seem this large, the maximal extension possible (to a value somewhat above 180 degrees) would fall far short of supplying the 4-fold increase needed at 100 mm. Hg. For this reason it is apparent that the necessary changes in stroke volume must be effected in part through alteration in the angle of attack.

Since the stroke volume will vary with the sine of α, which for small values of the angle changes approximately as the angle itself, the hypothesis seems acceptable that the insect utilizes this mechanism in partial compensation for changes in air density. Lacking information to the contrary, we may conjecture in analogy with larger airfoils that the insect wing operates most efficiently under normal conditions with small values of α in the range from 0 degrees to 5 degrees, which are increased at densities lower than normal and reduced at higher densities. The same mechanism is familiar, of course, in the variable-pitch propellors of modern aircraft.

In summary, then, we may state that the wingbeat frequencies observed at densities other than normal are understandable only in terms of simultaneous variation in another element of the wing movement. A survey of the possibilities suggests that this must be the stroke volume, and that a part of the compensation derived from this source may be attributed to alterations in stroke amplitude, a reduction of which has been observed but not measured at higher densities. The larger share of the necessary variation in stroke volume comes apparently from small changes in the angle of attack. These have not been measured nor, since the direction of the relative wind must vary continuously as the wing sweeps through its complex path, does it seem likely that they will be. Power output and input, and the overall efficiency linking them, are probably essentially independent of variations in density.

The fact that the strain which results from alteration in the stress imposed by air resistance is distributed over several elements in the wing movement suggests analogies between the latter and other physiological functions in which homeostasis is observed. Wingbeat frequency changes less with alterations in density than if it alone were to compensate, while the deduced changes in stroke volume appear to be shared between alterations in amplitude and variations in the angle of attack. With regard to wingbeat frequency we know that it is governed largely by such physiological and environmental factors as substrate concentration (Williams, Barness and Sawyer, 1943), temperature, age and sex and we may imagine that these set a tempo of neuromuscular activity from which the organism has difficulty in departing even when confronted with major variations in other external influences. The way in which stroke amplitude and angle of attack may be regulated is unknown, but their dependence on density suggests the possibility of reflex control, mediated perhaps by campaniform receptors on the wings in response to variations in the amount of bending caused by air resistance.

SUMMARY

Wingbeat frequency of *Drosophila repleta* Wollaston was measured stroboscopically at 25 degrees C. as a function of atmospheric pressure, over the range from 100 mm. Hg to 3860 mm. Hg in air, in nitrogen-oxygen mixtures containing either more or less oxygen than air, and in two helium-oxygen mixtures. Similar measurements were made with *D. virilis* Sturtevant at 19.3 degrees C. and 25.9 degrees C. in air over the pressure range from 100 mm. Hg to 1520 mm. Hg; and at 760 mm. Hg, 25.9 degrees C., in a mixture of 6.1 per cent oxygen in nitrogen.

The flight response was inhibited when total pressure was less than 80 to 100 mm. Hg, or when the oxygen tension was less than 15–20 mm. Hg. Increasing the partial pressure of oxygen above the value for air did not increase the rate of wingbeat.

Within the limits of experimental error, the rate was found equal at equal densities, irrespective of the medium in which it was measured. Wingbeat frequency is therefore independent of total pressure as such, and varies inversely in a logarithmic relationship with the density. The exponents measured for this relationship varied with different individuals between − 0.03 and − 0.15, approximately.

The helium-oxygen mixtures had a detrimental effect on the response of the insects, which was less evident at higher pressures and reversed when the specimens were returned to air.

Amputation of the halteres did not disturb the relationship between wingbeat frequency and density.

Clipping portions from the wingtips increased the frequency of wingbeat. When only one wing was clipped, the increase was less than when both were shortened by equal amounts.

Oxygen consumption of *D. virilis* was measured during flight in an oxygen atmosphere at 19.3 degrees C., at 760 mm. Hg, 400 mm. Hg and 200 mm. Hg, and was found to be relatively unaffected by variation in density.

Since wingbeat frequency varied less rapidly with changes in density than would be expected if both power output and stroke volume were to remain constant, it is reasoned that partial compensation is effected through adjustments in stroke volume. A decrease in stroke amplitude was observed at higher densities, but it appears unlikely that amplitude can increase enough at lower densities to account for the stroke volume required. Arguments are given to show that the remaining compensation needed may be furnished by alteration within reasonable limits of the angle of attack.

It is concluded that insect flight exhibits homeostatic characteristics, in that the strain which results from density change is distributed over several elements in the wing motion.

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ACTION OF ACETYLCHOLINE, CARBAMINOYL–CHOLINE (DORYL) AND ACETYL–B–METHYL–CHOLINE (MECHOLYL) ON THE HEART OF A CLADOCERAN

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This paper deals with the action of acetylcholine, carbaminoyl-choline, and acetyl-B-methyl-choline on the heart of the cladoceran *Simocephalus vetulus*. Our interest in this work is primarily centered on the comparative physiological evaluation of these chemicals with regard to their stability, potency of action, and their pharmacological effects on the heart as compared with the effects on the intestine of this animal and *Daphnia magna* (Obreshkove, 1941; Mooney and Obreshkove, 1948). A correlation is also made of the observations presented in this paper with certain facts pertaining to the role which tese drugs have been said to play in the transmission of nervous impulses.

METHODS

In this study *Simocephalus vetulus* in their second instar were utilized. The method of rearing and selection of animals for the experimentation as well as the method employed in administering the drugs have been described elsewhere (Obreshkove, 1930, 1941). A single individual in each case was transferred to a micro-culture slide for treatment with the specific drug, and for examination of the activity of the heart under the microscope. After the transference of the animal to the slide, there was usually observed a slight excitation of the heart which persisted for about 15 seconds. One minute was therefore allowed to elapse before the actual readings were taken. Since the animal is seen at all times to ingest particles and fluid with which it comes in contact in the depression slide, it is assumed that the drugs employed in this work were administered orally. The rate of the heart beat was recorded on paper with penicil dots in synchronic rhythm with the heart contractions.

Acetylcholine

It was shown in the study of the effects of acetylcholine on the intestine of *Daphnia magna* and *Simocephalus vetulus* reported previously that when this drug becomes effective, it exhibits its action to the fullest extent with an abrupt, powerful, contractile wave of considerable amplitude. The vigorous peristaltic waves become further intensified with lapse of time, persist for several hours, and terminate in intestinal contracture. The effectiveness of the drug on the heart of *Simocephalus vetulus* is best expressed by the time required for the establishment of the maximum rate of contraction rather than by the time required for the production of an initial effect. In the heart, as in the intestine, there was demonstrated a graded action over a wide range of concentrations. Acetylcholine ranging in concentration from

1×10^{-2} to 1×10^{-9} was employed. In Table I are given the results obtained with 2 concentrations of the drug. It may be seen that when *Simocephalus vetulus* young are treated with acetylcholine, an increase in the rate of the heart beat is observed. Although prolonged consecutive readings were taken for each animal after the application of this and the other drugs employed, in a number of tables presented here, only the maximum heart rate was recorded. With acetylcholine 1×10^{-5} the first evidence of increased cardiac activity appears about 2 minutes after the application of the drug and a maximum rate is established in about 10 to 15 minutes. This is followed by a gradual decline in the heart beat to a subnormal rate which persists for some time. Baylor (1942) also reports a depressing effect of acetylcholine on the heart rate of *Daphnia magna* some minutes after the applica-

TABLE I

Effect of acetylcholine 1×10^{-5} and acetylcholine 1×10^{-2} on the heart rate of Simocephalus vetulus

Normal rate	Acetylcholine 1×10^{-5}		Normal rate	Acetylcholine 1×10^{-2}	
Beat/min.	Min.	Beat/min.	Beat/min.	Min.	Max. beat/min.
244	2	242	274	5	314
	4	260			
	10	296*			
	15	200			
261	2	282	276	5	314
	5	270			
	10	293*			
	15	207			
277	5	286	264	6	303
	10	295			
	15	309*			
	20	278			
276	4	288	268	5	302
	10	299			
	12	303*			
	15	286			
280	5	278	270	5	318
	10	305*			
	15	194			
275	5	310	272	3	300
	10	301			
	15	315*			
	20	224			
284	5	298	274	4	304
	10	314			
	13	316*			

* Max. rate.

tion of the drug but failed to observe the acceleratory action described here. Stronger solutions of acetylcholine appreciably reduce the time required for the production of the maximum cardiac activity in *Simocephalus vetulus.* Acetylcho-

TABLE II

Effect of prostigmine 1×10^{-4} *when employed alone and the effect of acetylcholine* 1×10^{-5} *when preceded by 2 minutes treatment with prostigmine* 1×10^{-4} *on the heart rate of Simocephalus vetulus*

Normal rate	Prostigmine		Normal rate	Acetylcholine 1×10^{-5} after 2 min. treatment with prostigmine 1×10^{-4}	
Beat/min.	Min.	Max. beat/min.	Beat/min.	Min.	Max. beat/min.
230	20	268	284	2	321
261	15	294	275	2	320
290	15	319	278	2	325
271	20	329	260	2	306
263	15	298	273	2	309
266	15	294	269	2	297
278	20	308	272	2	303
268	20	299	265	2	294

TABLE III

*Effect of mecholyl** 1×10^{-5} *on the heart rate of Simocephalus vetulus following atropine* 1×10^{-5}

Normal rate	Atropine 1×10^{-5}		Mecholyl 1×10^{-5}	
Beat/min.	Min.	Beat/min.	Min.	Beat/min.
272	5	271	2	272
			7	271
			10	269
			30	265
261	5	266	1	262
			3	265
			12	266
			30	262
264	5	263	2	260
			5	262
			10	266
			22	256
286	5	282	2	281
			5	282
			10	278
			15	285
276	5	275	2	282
			4	261
			6	280
			8	268

* Mecholyl—trade name for acetyl-B-methyl-choline.

line 1×10^{-2} produces the maximum effect in a period which varies from 3 to 6 minutes as compared with the 10 to 15 minutes required by acetylcholine 1×10^{-5} (Table I). When acetylcholine (1×10^{-5}) is preceded by prostigmine (1×10^{-4}) the maximum effect appeared in about 2 minutes (Table II) in comparison with the 10 to 15 minutes when acetylcholine 1×10^{-5} was employed alone. A similar action was demonstrated for physostigmine in the production of an intensification of the Doryl and Mecholyl effects. The observations are in accord with those made on the intestine of this animal (Mooney and Obreshkove, 1948). Likewise when prostigmine was administered alone, it produced the same effect as acetylcholine but required on the average a longer period of time for the production of the maximum effect (Table II).

TABLE IV

Effect of Doryl on the heart rate of Simocephalus vetulus; abolishing of this effect by atropine; and reestablishment of the Doryl effect*

Normal rate	Doryl 1×10^{-10}		Atropine 1×10^{-5}		Doryl 1×10^{-5}	
Beat/min.	Min.	Maximum beat/min.	Min.	Beat/min.	Min.	Maximum beat/min.
284	3	323	2 3	271 273	3	301
261	2	290	2 3	263 265	3	284
268	4	287	1 5	266 251	3	293
265	3	290	2 3	250 253	3	293
273	1	295	2 4	282 270	3	288
281	3	296	1 2	261 263	3	304
281	3	308	2 5	304 270	3	292
269	1	298	2 5	283 260	1	288
276	3	304	1 4	297 275	2	299
266	3	304	1 4	285 271	2	298

* Doryl—trade name for carbaminoyl-choline.

Acetyl-B-methyl-choline (mecholyl)

Acetyl-B-methyl-choline exerts a characteristic effect on the heart of *Simocephalus vetulus* in eliciting a cardiac acceleration in a manner similar to that of acetylcholine. It appears that this drug is more potent on the heart than acetylcholine as judged by the time required for the production of the maximum excitatory action when drugs of the same concentration are employed. A very clear antagonism was found to exist between acetyl-B-methyl-choline and atropine. In a series of 5 experiments a preliminary application of atropine 1 × 10^{-5} for 5 minutes prevented the appearance of the characteristic effect ascribed to Mecholyl even 30 minutes after the application of the latter drug (Table III). The same concentration of atropine was found effective when added after Mecholyl. These observations are similar to those reported for the intestine of this animal.

TABLE V

Effect of Mecholyl 1 × 10^{-5} on the heart rate of Simocephalus vetulus and the abolishing of this effect by atropine 1 × 10^{-5}*

Normal rate	Mecholyl 1 × 10^{-5}		Atropine 1 × 10^{-5}	
Beat/min.	Min.	Max. beat/min.	Min.	Beat/min.
260	3	293	2 3	264 258
277	3	302	1 3	275 278
289	3	308	1 3	284 286
282	4	306	1 2	283 280
271	1	305	1 3	284 280
268	1	301	2 3	267 270
270	2	316	2	278

* Mecholyl—trade name for acetyl-B-methyl-choline.

Carbaminoyl-choline (doryl)

The heart of *Simocephalus vetulus* responds even to a very weak solution of carbaminoyl-choline. A maximum rate of heart beat is produced by a low concentration of this drug (1 × 10^{-10}) in a comparatively short period of time. Whereas atropine was shown to abolish rapidly or prevent completely the action of acetyl-B-methyl-choline, when the doryl effects are abolished by atropine, they are quickly reestablished after the atropine treatment is followed by carbaminoyl-choline (Table IV). Prolonged washing reduces and in time completely abolishes the effects of carbaminoyl-choline and of all the other drugs employed in this work. In abolishing the action of the drugs, the effects of washing were found to be considerably slower than those produced by atropine.

TABLE VI

Action of Doryl 1 × 10^{-5} followed by washing with distilled water on the heart rate of Simocephalus vetulus (second day young)

No.	Normal	Doryl 1 × 10^{-5}%		Distilled H_2O	
	Beats/minute	Minutes	Beats/minute	Minutes	Beats/minute
1	238	2½ 5	289 285	2 5 10 15 20	281 279 258 257 250
2	256	2½ 3½	294 295	2 4 6 10 15	290 291 289 276 270
3	282	2 2	301 307	2 4 10 15	304 300 287 288
4	277	2 3	298 301	2 4 10 15	299 301 285 272
5	269	2 3½	296 303	2 4 10 15	294 291 274 270

DISCUSSION

Our endeavor to demonstrate the nerves distributed to the heart of *Simocephalus vetulus,* so important for the further clarification and analysis of the results presented here, was beset with many difficulties. It was demonstrated earlier, however, that if the intestine of *Daphnia magna* is touched with a fine glass needle at the bend of the digestive tube where the stomach enters the intestine, the heart immediately ceases to beat and after a certain period, depending on the degree of the mechanical stimulation applied, the heart escapes from inhibition (Obreshkove, 1942). The heart in this respect behaves similarly to the inhibition produced after electrical excitation of the vagus nerve in vertebrates. When *Daphnia magna* was treated with acetylcholine, prior to the production of inhibition or during the heart inactivity, the period of cardiac inhibition was observed to be considerably shorter. It must be pointed out that whereas acetylcholine has been shown to produce cardiac excitation in some invertebrates, in others it produces inhibition. Prosser (1942) after an extensive review of the literature presents evidence to indicate that the invertebrate hearts may be grouped into three classes with respect to the action of acetylcholine and suggests that those hearts which are accelerated by the drugs are neurogenic, those which are inhibited are myogenic and those which are unaffected by acetylcholine are non-innervated.

Acetylcholine, acetyl-B-methyl-choline, and carbaminoyl-choline produce cardiac excitation in *Simocephalus vetulus.* This action of the drugs is antagonized by atropine and augmented by prostigmine and physostigmine. These and the other observations recorded in this paper are of such a nature as to suggest strongly some known facts pertaining to the role which acetylcholine and other cholinergic drugs have been said to play in the transmission of nervous impulses. The demonstration by Artemov and Mitropolitanskaja of an acetylcholine-like substance in Daphnia (1938) adds further interest to the problem.

SUMMARY

1. Acetylcholine, carbaminoyl-choline and acetyl-B-methyl-choline produce in *Simocephalus vetulus* cardiac excitation. The heart acceleration in each case was followed by inhibition.

2. This action of the drugs is antagonized by atropine and augmented by prostigmine and physostigmine.

3. Acetylcholine exhibits a graded action for a wide range of concentrations (1×10^{-2} to 1×10^{-9}) as revealed by the time required for the production of a maximum cardiac excitation.

4. In contrast with carbaminoyl-choline, a marked antagonism was found to exist between acetyl-B-methyl-choline and atropine. When atropine is followed by carbaminoyl-choline, the Doryl effect appears in comparatively short periods of time.

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A CYTOTOXIN FROM BLEPHARISMA

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When a few paramecia were added to a concentrated suspension of *Blepharisma undulans* in a Cartesian diver, they were injured, began to rotate, and after swelling, died, although the Blepharisma remained normal and active (Giese and Zeuthen, 1949). A few individuals from a Blepharisma culture were placed with a lot of paramecia with no ill effect. An attempt was made to determine what caused the injury to Paramecium placed in a concentrated culture of Blepharisma. The results are described below.

EXPERIMENTAL

Cultures of Blepharisma were grown as previously described (Giese, 1938b). Practically all the other organisms were grown in lettuce infusions of the same type (0.05 per cent lettuce, buffered at pH 7.0 or 8.0), or obtained from wild cultures. *Paramecium multimicronucleatum* for division studies was grown as previously described (Giese, 1945).

In the first experiment the culture of Blepharisma was handled with great care and the animals were gently centrifuged down into the cone of a centrifuge tube. The supernatant was carefully withdrawn and after a dense suspension was available, some paramecia were added. They were in no way adversely affected. It was therefore apparent that when Blepharisma individuals are handled with care they do not liberate any substance injurious to Paramecium. The inference may be drawn that in the pipetting of the suspension of Blepharisma into the diver some individuals may have been injured. To test this possibility individuals in a dense culture of Blepharisma were fragmented by sucking the animals up into a pipette partially blocked by cotton fibers, making a "brei." In this process the animals were torn open and the fluid became pinkish.

Paramecia added to the brei reacted violently by reversed ciliary action and then quickly began moving and died. In a freshly prepared brei, the time from immersion to killing was only a few minutes. A Paramecium-brei similarly prepared was not toxic to Blepharisma nor was a Didinium-brei toxic to Paramecium. Therefore Blepharisma presents a special case worthy of further study.

Questions arise as to the nature and properties of the material liberated by Blepharisma (hereafter called the toxin without any implications other than that it is a poison of organismal origin). It is desirable to know whether the toxin is

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* I am indebted to Dr. L. Garnjobst of Stanford University for a culture of Actinosphaerium, to Dr. W. H. Johnson of Wabash College for a culture of Woodruffia, and to Dr. Frederick Evans of the University of Utah for a culture of Podophrya. I am also pleased to acknowledge the skillful assistance of Mrs. Helene Leighton in the experiments on the rate of growth of Paramecium in the presence of Blepharisma and to Miss Eugenia Brandt for repetition of a number of experiments on the effects of the toxin upon a number of protozoons.

selectively injurious to Paramecium or whether it is generally toxic to organisms. Secondly, the possible function of this toxin is also of interest. Thirdly it is desirable to identify the toxin with some cellular constituent. Experiments attempting to answer these inquiries are described below.

To determine if the toxin liberated by Blepharisma is specific to Paramecium or generally injurious, a wide variety of protozoa were tried. In no case were the protozoans found to be resistant to the Blepharisma-extract, although some were more susceptible than others. *Frontonia leucas* was found to be very susceptible, and it and *Urocentrum turbo* were more susceptible than *Colpidium colpoda* placed in the same brei. The latter was more susceptible than *Paramecium multimicronucleatum* and *P. aurelia. Paramecium bursaria* (green), *Stylonychia curvata, Euglena gracilis, Amoeba proteus* and *Actinosphaerium eichhorni* were found to be more resistant that *P. multimicronucleatum.* Even Rotifers were observed to be affected. Not alone are infusorians injured. Blastulae and gastrulae of the sea urchin, *Stronglyocentrotus purpuratus,* were exposed to small amounts of the substance. They ceased swimming and did not recover; in a few hours they had disintegrated. The material liberated by fragmented Blepharisma individuals seems to be a rather general cellular toxin.

To determine whether the toxin were liberated in limited quantities, a succession of additions of paramecia was made and it was found that whereas the second batch was readily killed, thereafter, the time for killing increased until after many additions there appeared to be no injury. The material appeared to be adsorbed or absorbed by the paramecia and so removed from the solution.

Attempts were made to wash paramecia free of the toxin when they had shown only the first signs of injury, for example, reversed ciliary activity. In no case was the injury reversible, but became more and more pronounced until the paramecia died. Therefore, the toxin seems to become firmly attached.

To determine whether the toxin is injurious to Blepharisma itself, individuals were exposed to a freshly prepared brei. They were not affected and, in fact, they began to clean up the fragments of the corpses as could be seen by the deep red vacuoles within them. Some become giants (see Giese, 1938b, for an account of gigantism in this species). They seem unaffected by having the smaller fragments of their fellows inside them and the toxic material outside. Division was observed to occur and a healthy culture was established. Since many very minute living individuals were also observed in a culture fragmented by passage through cotton fibers, it seems likely that some of the fragments regenerate. The conclusion may be drawn that the material liberated by fragmented Blepharisma while toxic to other forms, is not toxic to itself.

Since the exudate of Blepharisma is so toxic one wonders whether it functions in preventing attack by other organisms. In that case one might expect that carnivorous protozoans would avoid attacking Blepharisma. To test this, carnivores were placed with Blepharisma. *Didinium nasutum,* a particularly voracious ciliate, which attacks Paramecium and Colpidium, avoids Blepharisma. Didinium will starve to death in the midst of a rich culture of Blepharisma but also ignores such colorless forms as Stylonychia. Another ciliate, *Woodruffia metabolica,* also attacks *Paramecium* but ignores Blepharisma as well as many other ciliates. Also the suctorian *Podophrya fixa* feeds upon Paramecium and Colpidium but starves in a culture of Blepharisma. At about the time when it appeared likely that no carnivores would eat Blepharisma, Actinosphaerium was tried. Not only did this heliozoan feed upon Blepharisma but it did so voraciously and individuals of the latter were not only engulfed but digested. Almost as soon as a suspension of Blepharisma was added some were caught in the extended axopodia of the heliozoan. Sometimes on struggling they succeeded in breaking loose, but more often they did not. Within a few minutes they were engulfed in the streaming protoplasm and enclosed in a vacuole which was drawn towards the body. After several hours some individuals of Actinosphaerium had as many as twelve deep red vacuoles. After several more hours they were surrounded with red fecal deposits.

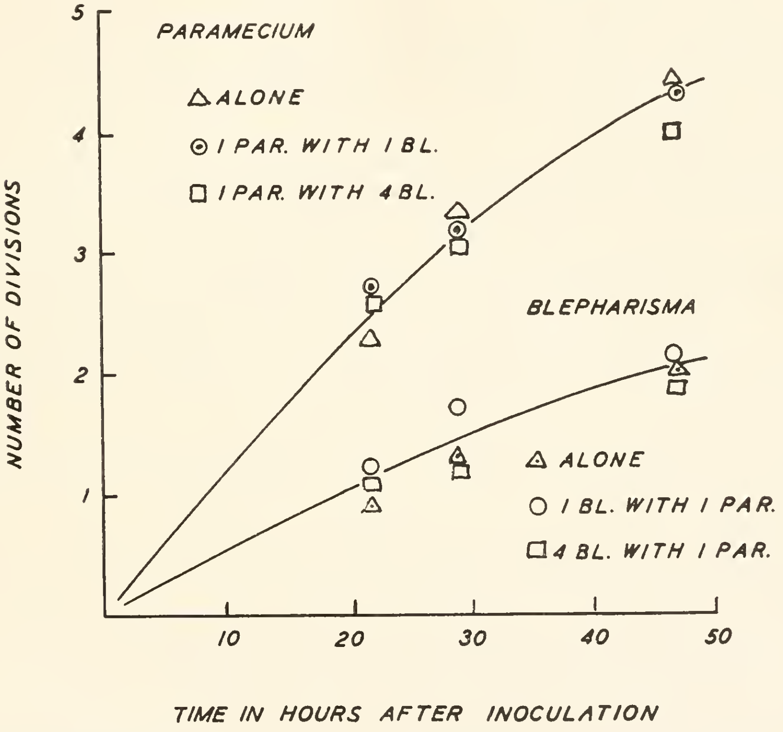

FIGURE 1. Comparison of division-rates of Paramecium in the presence and absence of Blepharisma. Three sets of eight cultures each were used for these determinations. In addition another series in which two instead of four Blepharisma were studied and still another with eight Blepharisma. All the experiments indicated the same result.

If one observes the food vacuoles within an Actinosphaerium, one will see that the contents decrease in size and become more intensely colored. As digestion proceeds the vacuoles do not seem to change hue, always appearing red. Sometimes the fluid within the vacuole turns pink. However the protoplasm of Actinosphaerium never takes on a reddish color. Actinosphaerium fed on Blepharisma continues to grow and divide. Whether division would go on at the same rate as on other food could not be determined since division occurred in such an erratic manner. The latter is probably due to the fact that Actinosphaerium is multinucleate and may grow to a large size before dividing. The experiments with Actinosphaerium demonstrate that in spite of its toxin, Blepharisma is not necessarily protected from carnivores.

Another possible function of the toxin in Blepharisma suggests itself for testing. Perhaps the toxin excludes other species of animals when it accumulates in a culture during growth. This could be tested by growing Blepharisma together with another species in the same culture. Such experiments were performed with Paramecium and Blepharisma. A single specimen of *Paramecium multimicronucleatum* and of Blepharisma placed together in a tube of culture medium grew at about the same rate at controls grown separately. A single specimen of Paramecium placed with four individuals of Blepharisma also grew as well as the control in the absence of Blepharisma. The data are summarized in Figure 1. The conclusion may be drawn that if something is exuded from Blepharisma during growth it is insufficient to prevent paramecia from growing at least as rapidly as they would in the absence of Blepharisma.[1]

The possibility that the toxin might be the reddish pigment suggests itself since in experiments on the effects of the brei on paramecia and some of the other colorless forms it was noted that the animals became reddish after they were injured. If this were true then if the pigment were first destroyed by bleaching one might expect the brei of such animals to be innocuous. Accordingly two experiments were tried. In the first the individuals in a culture of Blepharisma were killed and disrupted by exposure to visible light (for method see Giese, 1946) and the light treatment was continued until relatively little color remained. To this material, paramecia were added and it was found that there was little if any observable effect on them. In the second set of experiments the culture of Blepharisma was first bleached by exposing it to weak light (Giese, 1938a). This was accomplished by placing it near a 6-watt daylight fluorescent lamp cooled by a fan. From the animals bleached for 24 hours a brei was made and it proved completely non-toxic to Paramecium. The material which is toxic is therefore photolabile. However the pigment might merely act as a photosensitizer to some other constituent such as a protein or fat of the cell which when affected becomes toxic. This might be answered by separating the pigment from the fats and proteins of the cell.

The pigment was next extracted with absolute alcohol (Emerson, 1935) from animals concentrated into a small red mass by centrifuging. It was then freed from particulate detritus by centrifuging and dried in a water bath and was reextracted with absolute alcohol and again dried in another dish. It was then extracted with water. Only a portion of the original pigment went into aqueous solution which was clear and reddish. From the solubility properties it would appear that the toxic substance of Blepharisma is not related to the killer substance paramecin (Sonneborn, 1948; van Wagtendonk, 1948) produced by some strains of *Paramecium aurelia*.

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[1] One unexpected result of growing Paramecium and Blepharisma together is the formation of Blepharisma giants which eat Paramecium. This occurs only in cultures with *P. aurelia;* at least it was never observed in the cultures with *P. multimicronucleatum.* It is probable that the latter species is just too large to be engulfed since even when specimens of Blepharisma had become very much enlarged as a result of feeding on smaller species, they did not succeed i ingesting the larger species of Paramecium.

Specimens of Paramecium introduced into diluted aqueous pigment solution reacted much as they did to the crude material from crushed Blepharisma. They showed very strong reversed ciliary activity, then began to rotate; and as the contractile vacuoles ceased working, the animals became enlarged and died. Upon dying they became distinctly stained with a reddish tinge. While it is not certain that something toxic is not combined with the pigment, such preliminary trials as have been made using absorption column analysis indicate a single substance. The tentative hypothesis is put forth that the pigment is the toxic material. This can only be tested further by purification and study of the pigment. Such experiments are under way.

SUMMARY

1. A brei of fragmented Blepharisma contains some substance which is quite toxic to Paramecium and a variety of other protozoans and to sea urchin larvae, but it is not toxic to Blepharisma itself.

2. Paramecia suspended in a dense culture of Blepharisma are unaffected by the mere presence of Blepharisma.

3. Blepharisma is eaten by Actinosphaerium; therefore the toxin does not protect it from attack and use as food, but it is not eaten by Woodruffia, Podophrya or Didinium.

4. In the presence of Blepharisma, paramecia grow at the same rate as they do alone, indicating that no toxin is secreted during growth.

5. Brei of Blepharisma bleached by light is not toxic to paramecia.

6. The pigment of Blepharisma extracted in alcohol and after drying re-extracted in alcohol and, after another drying, re-extracted in water is highly toxic to paramecia.

7. The tentative conclusion is drawn that the toxin is the pigment or something very closely associated with it.

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VIABILITY AND FERTILITY OF DROSOPHILA EXPOSED TO SUB–ZERO TEMPERATURES[1]

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INTRODUCTION

The dependence of the effects of x-irradiation on various physical variables has provided an effective tool bearing on the problem of chromosome breakage and gene mutation induced by such irradiation. The study of one of these variables, temperature, is obviously limited to the range which the organism can survive. This is particularly true of a large multicellular animal such as Drosophila. Since in general low temperatures do not have detrimental biological effects unless ice crystals are produced and since the freezing point of many cells is far below what would be predicted from their osmotic pressure (Luyet and Gehenio, 1940), it seemed likely that one could extend the temperature range of experiments on Drosophila most effectively by exploring the effects of temperatures lower than 0° C. The design of the experiments to be described was dictated by the conditions of the typical x-ray treatment; certain aspects of the broader problem of viability and fertility under these conditions have therefore been emphasized, others ignored.

MATERIALS AND METHODS

Equipment

Treatment was made in a specially designed cold temperature chamber, consisting of an insulated box of about two cubic foot capacity cooled by coils from a $\frac{1}{3}$-horsepower refrigeration unit and heated simply by a hundred watt lamp. A partition divided the chamber into two sections, the upper of which was used for treatment, while the lower contained the heating and cooling units. A $\frac{1}{70}$ horsepower blower at one end of the partition forced the air from the lower into the upper chamber; an opening over the refrigerator coils at the other end provided for the free circulation of the air. A thermostat was placed in the blower air blast from the lower section into the upper one. Thus a heating-cooling cycle was completed about every half minute. Thermocouple measurements showed that the air in the upper chamber varied during the cycles not more than $1\frac{1}{2}$° C. on either side of the average temperature measured with a standard mercury thermometer. In those cases where the temperature variation was to be minimized the material was placed in a 15 cubic inch cardboard box which could be closed after the desired temperature had been reached in the chamber. The variations in the cardboard box, again meas-

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ured with a thermocouple, amounted to only ten per cent of those in the surrounding air blast. Cellophane windows were provided in both the removable cork top of the chamber and in the small box to facilitate checking the material during treatment and to minimize the absorption of X-rays during irradiation.

Rate of change of internal temperature

Thermocouple measurements inside the thorax of the fly subjected to an air blast at − 8° C. indicate that the internal temperature of the fly drops at an initial rate of about 1.6° C. per second, the rate becoming less as the gradient between the external and internal temperatures decreases, and that the fly and air temperatures are the same in from two to three minutes. The authors are indebted to Professor A. C. Fabergé who constructed by intricate plating techniques a very fine thermocouple for the purpose of making these measurements.

Genetic methods

The choice of stocks used in this work was determined by the plan of concurrent irradiation experiments; therefore the highly inbred Canton-S strain of *Drosophila melanogaster* was used. In fertility tests, these flies were provided with mates from the "Muller-5" strain which is now widely used in tests of irradiation effects in this species. The individuals to be treated were placed in size 00 gelatin capsules with holes punctured in both ends for rapid ventilation. Usually twenty flies at a time were placed in one capsule; numbers presented in the viability experiments therefore occur in approximate multiples of twenty which represent grouping of individual runs. Tests of fertility were made by placing single treated individuals with appropriate mates in 8 dram shell vials containing standard cornmeal-molasses-agar Drosophila culture medium.

Results

General behavior at low temperatures

As the internal temperature of the fly drops, it becomes sluggish and at + 3° C. all movement stops. A normal posture is then maintained regardless of the extent of the subsequent decrease in temperature. Lethality manifests itself during the period of recovery from the cold treatment. If the treatment is too severe, the fly assumes a posture characteristic of death by overetherization, i.e. the wings are held parallel in an upwards position. In some instances sub-lethal temperatures have impaired the locomotor control of the flies. Such individuals, after removal to room temperature, remain motionless or make feeble and uncoordinated attempts to walk. One such ataxic individual remained alive for 3 days; but for the purpose of this work such cases will be included with the deaths.

Effects of pretreatments

In the earliest series it became obvious that Drosophila under the influence of ether were particularly sensitive to the cold shocks. In the experiments to be described, the individuals were always allowed to recover completely from etherization before treatment. The high sensitivity under these conditions may account for the failure of some workers (see the discussion) to use temperatures below 0° C. Other techniques were tried to increase the resistance to cold without effect; these include pretreatment with $CaSO_4$ desiccant, with temperatures less severe than the final one (1° C. for 20 minutes before exposing the fly to − 10° C.), and with a sudden exposure of the animals to a temperature lower (− 20° C.) than that of treatment.

Viability of Drosophila at low temperatures

At 0° C., Drosophila males can survive for about 24 hours; since they die in about the same time in isolated capsules at room temperature, no additional work has been done in this range. The percentage of mortality for various durations of exposure at − 5° C., − 10° C., − 15° C. and − 20° C. are given in Table I. At − 5° C., two hours duration does not kill 100 per cent of the flies; at − 10° C., 25 minutes treatment is completely lethal; at − 15° C., 14 minutes is lethal and at − 20° C., 2 minutes. The thermocouple measurements previously referred to suggest that in all series except the last, the time required for the flies to reach the temperature of the chamber is insignificant; in the last, however, some temperature between − 15° C. and − 20° C. is lethal per se without respect to time duration. One set at − 10° C. run at 2 minute intervals from 14 to 32 minutes, with parallel sets of males and females showed identical sensitivities of the two sexes.

TABLE I

Mortality of Drosophila melanogaster males treated for various durations of time at 4 subzero temperatures. A = duration of treatment in minutes; B = number of males treated; C = number killed by treatment; D = percentage of mortality.

−5° C.														
A	10	20	30	40	50	60	70	80	90	100	110	120		
B	20	40	60	40	20	60	80	82	80	40	20	40		
C	1	0	1	1	0	3	5	41	54	2	11	30		
D	5	0	1.6	2.5	0	5	6.3	50	68	5	55	75		
−10° C.														
A	10	20	21	22	23	24	25	26	27	28	29	30	40	50
B	60	178	60	60	20	60	20	60	20	60	20	237	80	20
C	6	27	1	1	2	22	20	59	20	60	20	228	80	20
D	10	15.2	1.6	1.6	10	36.7	100	98.4	100	100	100	96.2	100	100
−15° C.														
A	1	2	3	4	5	6	7	8	9	10	11	12	13	13
B	20	20	20	20	40	20	40	40	20	20	20	20	20	180
C	1	7	9	4	16	3	15	23	16	14	17	13	15	180
D	5	35	45	20	40	15	38	58	80	70	85	65	75	100
−20° C.														
A	.5	1	1.5	2										
B	20	20	20	20										
C	2	13	16	20										
D	10	65	80	100										

The sterilization of fertilized females

Preliminary tests of 32 females subjected to − 10° C. for one-half hour and subsequently mated had shown their fertility to be unimpaired. On the other hand, about 200 fertilized females exposed to − 5° for 56 minutes during an irradiation experiment proved to be sterile. Since, in the latter case, mature sperm as well as ova were subjected to the treatment, it appeared likely that the sterility was caused by an inactivation of the sperm stored in the female, or "desemination" (Muller, 1944). A number of different kinds of tests were made to determine whether the adverse effect of the low temperatures was on the ova or sperm carried by the fertilized females. In these runs, Canton-S females were placed in quarter pint milk bottles with Canton-S males for three days or longer in order to insure the insemination of most of them, the proportion fertilized being determined by a test of a sample of them made as a control. After treatment, they were placed individually in shell vials with food, and their offspring counted nineteen days later, unless otherwise noted.

TABLE II

Fertility of fertilized Drosophila females exposed to −5° C. and −10° C. for varying durations of time

Duration	Females treated	Females fertile	Offspring per fertile female	Females producing only one offspring
Temperature = −5° C.				
0	25	24	79	0
15	50	1	88	11
30	50	1	88	10
45	50	2	43	13*
60	50	1	100	8
75	50	0	—	8
90	50	0	—	6
Temperature = −10° C.				
0	25	24	92	0
5	50	0	—	12
10	36	0	—	4
15	50	0	—	6
20	50	0	—	5

* Includes one female which gave 2 offspring and another which gave 4.

Out of 50 fertilized females exposed to 0° C. for 6 hours, 7 were sterile; the untreated control showed 4 sterile out of 50. This treatment is ineffective in sterilization. A larger series was subjected to − 5° C. for periods from 0 to 90 minutes in 15 minute intervals and to − 10° C. for periods from 0 to 20 minutes in 5 minute intervals. The 0 minute series in each case represent the controls. The results are shown in Table II. It is obvious that − 5° C. does sterilize the females since only 5 out of 300 treated were fertile, whereas 24 out of 25 in the controls were fertile. The five cases of fertility after treatment at − 5° C. with durations from 15 to 60 minutes were cases of complete fertility. When the exposures were longer than 60 minutes, no completely fertile females were found. Likewise exposures to − 10° C. sharply decrease the percentage of fertile females. The sporadic production of single, or very few, offspring by treated females noted in Table II was probably overlooked in the earlier runs.

In order to determine whether there is any effect of the cold shocks in the germ line of the female, which would account for the above results, a series was run in which fertilized Canton-S females were mated, after exposure to a sterilizing dose, to Muller-5 males. In this way it is possible to differentiate between sperm stored in the female at the time of treatment which would produce round-eyed females progeny, and the sperm introduced by the Muller-5 males after treatment, which would give narrow-eyed female offspring. In the event of an effect on the germ line of the female, no offspring of either type would be anticipated. Progeny counts were made 13 days after treatment; this short egg-laying time should make more obvious any temporary sterilization of the female which a longer egg-laying period might obscure. The results are shown in Table III. The single female which produced after treatment offspring of the first insemination yielded only one, like the sporadic cases described above. The fertility of females treated and subsequently mated is, in this run, higher than that of the untreated females not mated afterwards. This difference may be due to an increased viability of offspring of the second mating. However, the essential point is that the germ cells of the female are apparently not affected by the treatment and that such sterilization as does occur must be attributed to the killing of sperm stored in the female.

TABLE III

The productivity of fertilized females exposed to −10° C. for 20 minutes and subsequently mated to Muller-5 males compared with that of females so treated but not mated subsequently and with untreated and unmated females

	Untreated not mated	Treated unmated	Treated mated
Total ♀ ♀ treated	23	11	79
♀ ♀ producing offspring of first insemination	11	0	1
♀ ♀ producing offspring of second insemination	0	0	47
Average no. of ♀ ♀ offspring per fertile female	18.6	0	33.5

Dissections of deseminated females and their untreated sisters as controls revealed no motile sperm in the ventral receptacles or spermathecae of the former, although there was an abundance of motile sperm in both these organs of the controls. In addition, the quantity of sperm (immotile) in the ventral receptacles of the deseminated females was much smaller than that in the controls, in most cases the receptacles appearing completely empty as if a contraction had expelled the sperm. The natural striations of the chitinous spermathecal wall prevented any comparisons of quantity in the two sets, although in a few cases where a spermatheca of a treated female had been broken by pressure, sperm appeared in approximately normal quantity; they were, however, immotile.

The sporadic occurrence of one or two offspring among deseminated females may have one of two explanations. Either the treatment is not inactivating all the stored sperm, or those few offspring result from eggs already fertilized, or in the process of fertilization, at the time of treatment. These alternatives have been differentiated in two ways. First if exposure to a deseminating dose kills all but small fraction of the stored sperm, then the application of two such doses should be effective in decreasing their incidence even more. Fifty fertilized females, subjected to two sterilizing doses of − 10° for 15 minutes separated by an interval of 2 hours at room temperature, produced 8 offspring, each from one treated female. Twenty-five untreated controls produced an average of 109.7 offspring in 24 fertile vials. This frequency of sporadics is of the same order of magnitude as that in the previous single shock treatments.

On the other hand, if the sporadic cases are to be accounted for by the presence of fertilized eggs in the oviduct of the female at the same time of treatment, then, since those eggs are laid first, the sporadic individuals should come primarily from the first eggs deposited. Once again 50 Canton-S females, presumably fertile, were treated with − 10° for 15 minutes and transferred to new culture bottles on 4 successive days. The eggs laid on the first day included 14 sporadic cases; those on the second, third and fourth, none. A similar run, interrupted after the second day, gave 8 sporadic cases in the first day, none on the second. The controls in both the above cases were highly fertile. It seems reasonable to conclude, then, that these occasional single progeny appearing after the cold treatments result from eggs which had been fertilized before the time of treatment.

Effects on fertility of the male

In marked contrast to the pronounced lethality of cold shocks on sperm stored in female Drosophila, spermatozoa in the males are more resistant to changes in temperature, although here, too, there appears to be some lethality. Thirty-eight males exposed to −10° C. for twenty minutes were all fertile. Their offspring appeared in the customary ten day period, which contradicts the possibility that the mature sperm were killed and that sperm differentiating after treatment were used.

TABLE IV

Sterility and productivity of Drosophila males exposed to low temperatures with and without 3600 r. of x-rays during a 56 min. interval

	Irradiated			Unirradiated	
	25° C.	0.5° C.	−5° C.	0.5° C.	−5° C.
Total treated ♂♂	65	102	115	100	100
Total fertile ♂♂	60	48	11	92	50
% fertile ♂♂	92.4	47.1	9.6	92	50
Number of female offspring/fertile male	30.1	11.8	9.4	22.4	22.4

Likewise 74 out of 158 (equals 47 per cent) of males treated with − 5° C. for one hour were fertile whereas a smaller untreated control series showed that 15 out of 19, or 78 per cent, were fertile. In all these cases, those males which produced any offspring at all produced the normal number. Comparable time-temperature series on fertilized females (see above) were almost 100 per cent effective in killing sperm. When males are dissected after treatment with an exposure that kills all the sperm stored in females, there appears to be no mortality of sperm in the testes or seminal vesicle.

In agreement with the observations of others (Medvedev, 1935; Mickey, 1939) that irradiation at low temperatures decreases the fertility of males to an extent greater than that anticipated on a single additive effect basis, the data in Table IV show the fertility and productivity of males (mated singly to 2 Muller-5 females in shell vials) after exposure to 0.5° C. and − 5° C., with and without a dose of 3600 r during a 56 minute treatment. In the unirradiated series, the percentage of males completely sterilized increases with decreasing temperature, but the number of F_1 female progeny (the males not being counted for technical reasons) from the fertile males is essentially normal under the conditions of the experiment. However, with irradiation not only does the percentage of fertile males drop more rapidly, but the number of female progeny of the fertile males is between a half and a third normal. This is apparently related in part, at least, to the greater production of chromosomal aberrations during irradiation, at the lower temperatures, which will be discussed in more detail elsewhere.

Discussion

Applicability of temperatures below 0° C.

From the results of the experiments described above, it is clear that Drosophila will tolerate somewhat lower temperatures than previous workers have used. Thus, there are a number of accounts in the literature of the use of low temperatures in the range of + 3° C. to + 15° C.; in a few cases 0° C. has been reached (Medvedev, 1935; Papalaschwili, 1935; Mickey, 1939; King, 1947) and there is one instance (Kerkis, 1939) where Drosophila has been subjected to a temperature of − 6° C. It seems clear that temperatures below 0° C. are generally applicable provided that care is taken to insure complete recovery from etherization before, and adequate ventilation during treatment. This may permit a decisive test of the hypothesis that the genetic effects of x-irradiation are the immediate result of ionization, since this hypothesis predicts that the results should be temperature independent.

Desemination of Drosophila females

In many types of experiments with Drosophila, one of the most burdensome chores is the collection of virgin females. The observation that sperm stored in a female may be killed by the application of low temperatures, without affecting the fertility of the treated females in subsequent matings provides an effective tool in Drosophila work. Briefly summarized the procedure adopted for this treatment is the following: From 50 to 100 etherized females are placed in one size 00 gelatin capsule which is ventilated by pin holes at both ends. After an hour or two, during which time the flies recover completely from the etherization, the capsules are placed in a cold air blast of − 10° C. for 10 minutes or of − 5° C. for 90 minutes. Upon removal from the low temperature, they may be mated immediately if their sporadic progeny are distinguishable genetically from those of the post-treatment mating, otherwise they should be kept in a culture bottle for a day before mating to allow them to deposit the few fertilized eggs unaffected by the treatment.

SUMMARY

1. At − 5° C. about 50 per cent mortality of Drosophila melanogaster is reached after two hours; at − 10° C. a 20 minute exposure kills very few whereas a 25 minute exposure is almost completely lethal; at − 15° C. about 50 per cent survive exposures less than 10 minutes long whereas an exposure of 13 minutes or longer is completely lethal; and at − 20° C. all individuals are killed within a few minutes.

2. Cold shocks of air at − 5° C. for 75 to 90 minutes and at − 10° C. for 5 to 20 minutes are lethal to sperm stored in adult females although such treatment has no effect on the subsequent fertility of such females. Males are not sterilized to any great extent by such exposures.

ACKNOWLEDGMENTS

The authors wish to express their thanks to their colleagues in the Departments of Zoology and Genetics of the University of Missouri, whose active interest made these observations possible, and in particular to Professor D. Mazia who contributed many helpful suggestions as well as necessary equipment during the course of the work.

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STUDIES ON MARINE BRYOZOA. IV. NOLELLA BLAKEI N. SP.

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Introduction

During the summer of 1946 while growing some *Perophora viridis,* a Protochordate, for use of students in the Marine Biological Laboratory (M. B. L.) invertebrate zoology course, the writer noted some small delicate Nolella colonies growing in the same culture dishes. Identification of the bryozoan species was difficult for two reasons: (1) because existing descriptions of various species of Arachnidium, Arachnoidea, and Cylindroecium or Nolella in all stages of their development are not as extensive as one might hope for and (2) the present specimens were studied in the living, growing state for only eight days, from August 25 to September 2, 1946; hence, only a few colonies could be observed and these mostly in the young, developing stage. Because of the desire to report the form so subsequent workers or collectors could watch for it and study it more fully it was deemed advisable to publish the following data on the species.

Collection and Growth Data

On August 14, 1946, the Marine Biological Laboratory supply department collected a quantity of *Perophora viridis* from Lagoon Pond, Martha's Vineyard, Massachusetts. The Perophora was conspicuously overgrown with *Aeverrillia armata* and hydroids. On August 16, some Aeverrillia sprigs and the greenest Perophora stolons and buds were selected and cut into 10 to 15 mm. lengths for culturing (as in Figs. 1, 2). The watch glasses with their taped colony fragments were immersed upside down in racks (Fig. 2) in large laboratory aquaria into which natural sea water was piped from a near-by bay. In time the Aeverrillia and Perophora fragments developed colonies with stolons radiating in several directions over the bottom of the glass (Fig. 1). These watch glasses were studied daily under the microscope. On August 25 Nolella stolons and bases were discovered in ten watch glasses. These were watched daily till September 2, when observations had to terminate. The specimens were identified as one of the Ctenostomata, family Nolellidae, genus Nolella, new species. It was finally named *Nolella blakei* in honor of a most esteemed professor and kindly adviser, Dr. Irving Hill Blake of the University of Nebraska.

Morphology of Nolella Blakei n. sp.

The young zoarium is soft, transparent, and inconspicuous. It consists of thread-like "stolons" and basally adherent, upright columnar zoids whose tips are squared. Very young zoids arise as squared peristomes from flattened, enlarged bases which have temporarily serrated borders.

"Stolons"

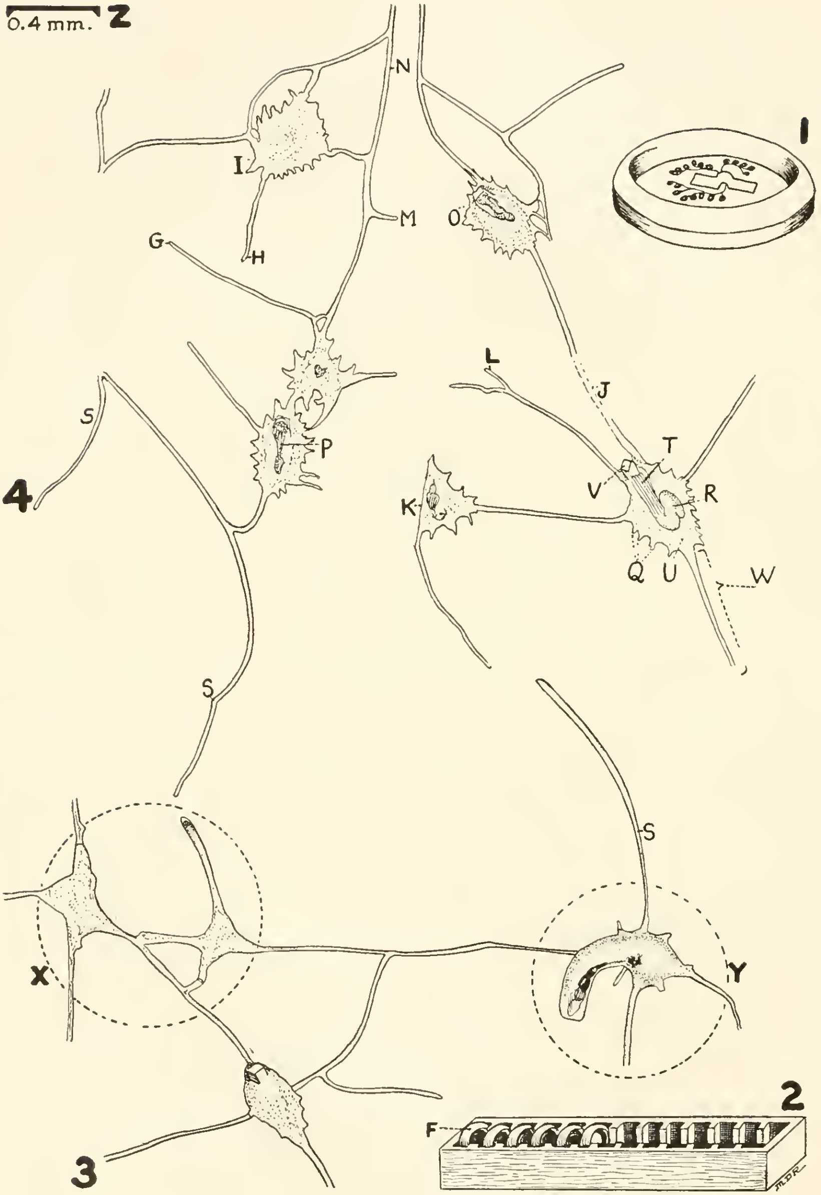
Annandale (1907, page 199), Harmer (1915, page 43), Silén (1942, page 6) and others questioned the suitability of the term "stolon" for the long creeping processes connecting the zoids of some Ctenostomes because these processes are really extensions of the zoid bases and are sometimes not closed off by a septum from the main body cavity of the zoid. See Figures 7, 12, 13, and 19 (W) for such stolons in young *Nolella blakei*. Some of the young *Nolella blakei* stolons have septa at their origin, others do not. The younger the colonies the more apt are they not to have yet formed septa. Whether there are some non-septate "stolons" in old *Nolella blakei* individuals is not known because the older zoids (Figs. 15, 17, 18) were torn from the taped Perophora so complete stolons were not generally obtained. The following description of *Nolella blakei* "stolons" is therefore based largely on conditions in young colonies. The "stolons" are thin-walled, slender (0.015 to 0.046 mm. in diameter), anastomosing, short or long (0.155 to 2.296 mm.) and adherent along their entire length. Three to five "stolons" originate from the zoid base (Fig. 5). They are generally separated by a septum at their proximal end or point of origin from the distal end of the basal enlargement (Figs. 7, 13). "Stolons" may grow into each other and establish new connections with other zooecia (see the solid black lines in Fig. 5) in a relatively short time (two days). Zoids arise as buds from the "stolons" (Fig. 10, A). The basal enlargement itself appears to have been formed at the distal end of a "stolon" from which it was not separated, at least in young colonies. Additional data on "stolons" and other parts are given in Table I.

TABLE I

Measurements of Nolella blakei n. sp.

	Maximum	Minimum	Average	No. of readings
Tentacle number	12	8	10	13
"Stolons"				
Length	2.296 mm.	0.155 mm.	0.767 mm.	35
Width	.046 mm.	.015 mm.	.029 mm.	33
Upright, retracted zoid:				
Length of vertical part, exclusive of proximal "stolon"	.899 mm.	.697 mm.	.775 mm.	3
Width	.093 mm.	.076 mm.	.049 mm.	3
Basal enlargement (exclusive of stolons):				
Length	.472 mm.	.328 mm.	.397 mm.	20
Width	.341 mm.	.205 mm.	.268 mm.	20
Number of small pointed extensions, exclusive of stolons, from the basal enlargement border, in young colonies	17	4	10	19

PLATE I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EXPLANATION OF PLATES

All figures except 1, 2, 6 and 13 were drawn with the camera lucida. All but Figures 1 and 2 are of the new species *Nolella blakei*. Figures 3, 7, 10, 11, 12 and 14 were drawn from colonies growing in one watch glass; Figures 4, 5, 8, 9, 16 from another and Figures 15, 17, 18 from colonies in a third watch glass.

Basal enlargement

The flattened basal enlargement of *Nolella blakei* is delicate, soft, transparent, and irregular in outline. It sometimes tends toward a rough diamond shape (Figs. 7, 8, 19). It is conspicuous in young colonies having a crenulate or serrate border in young zoids but is not noticeable in the few available older zoids (Figs. 15, 17, 18) because the growing zoid cylinder gradually incorporates it. From its sides extend outward five to seventeen serrate processes (Figs. 8Q, 16) which adhere to the substratum and are apparently only of a temporary nature. In time they become obliterated by the enlarging zoid. In *Nolella blakei* these cuticular projections are

TABLE II

Comparison of tentacle numbers in related species

Tentacle number	Species	Primary or secondary reference sources
12–16	*Arachnidium fibrosum*	Marcus, 1938, p. 51; 1941, p. 27
26–30	*Arachnidium irregulare*	Harmer, 1915, p. 49
16	**Arachnidium ray-lankesteri*	Rousselet, 1907, p. 255
18–20	*Arachnidium simplex*	Hincks, 1880b, p. 284
16	*Arachnoidea evelinae*	Marcus, 1937, pp. 130–131
16^+	*Arachnoidea protecta*	Harmer, 1915, p. 50
about 18–20	***Cylindroecium dilatatum*	Hincks, 1880a, p. 536
no data	*Cylindroecium horridum*	O'Donoghue, 1926, p. 61
about 10	*Cylindroecium pusillum*	Hincks, 1880a, p. 537
no data	*Cylindroecium repens*	O'Donoghue, 1923, p. 192
no data	*Cylindroecium spinifera*	O'Donoghue, 1924, p. 59
about 10	*Nolella alta*	Marcus, 1938, p. 55
16–20	*Nolella annectens*	Harmer, 1915, p. 59
8–12	*Nolella blakei*	present study
18–22	*Nolella gigantea*	Marcus, 1937, p. 132
about 18	*Nolella papuensis*	Harmer, 1915, p. 55

* Now Arachnoidea (Harmer, 1915, p. 51).
** Now Nolella (Marcus, 1938, pp. 53–55).

PLATE I

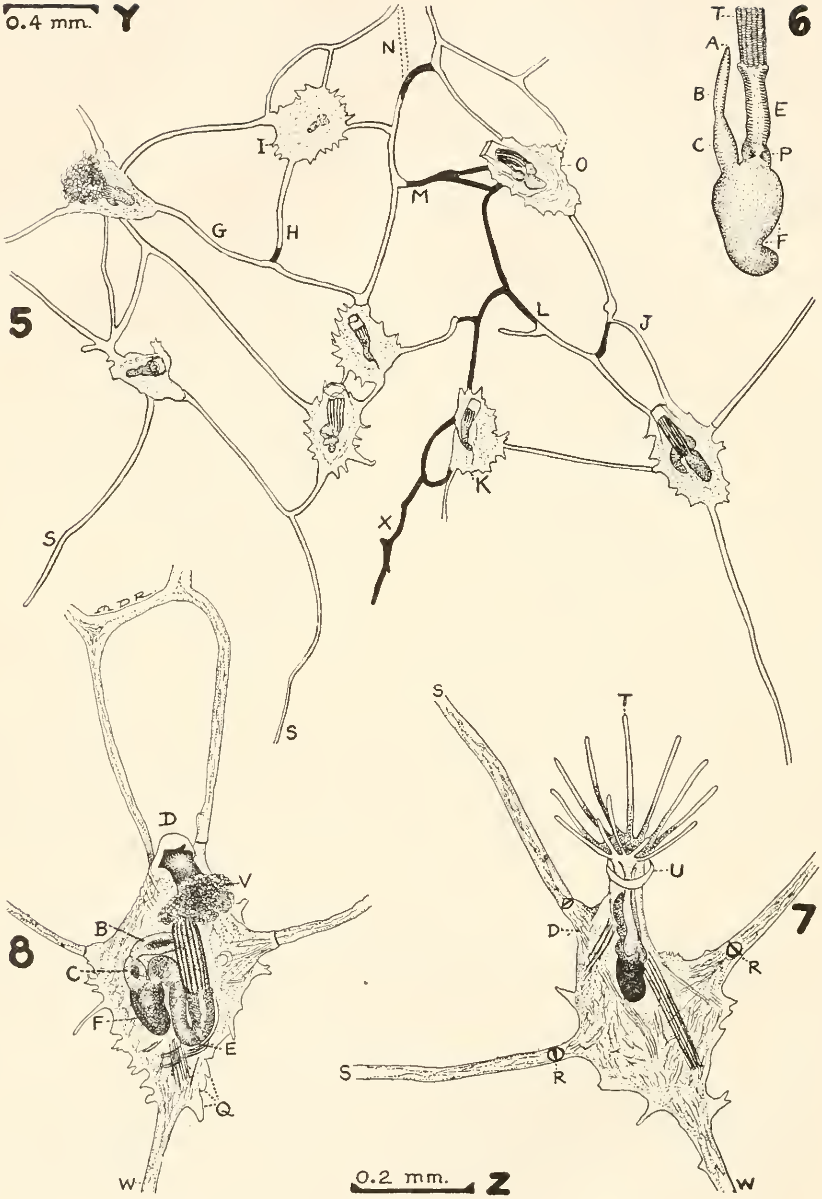
FIGURE 1. Diagram of a Perophora colony growing in a Syracuse watch glass from a fragment which had been fastened down with waterproof adhesive tape about one or two weeks before. Aeverrillia and Nolella grew under similar conditions along with the Perophora.

FIGURE 2. An open wooden rack containing several watch glasses (F) for culturing bryozoa and Perophora.

FIGURE 3. Part of very young *N. blakei* colony drawn on IX–1–1946. Circle X encloses two basal enlargements which as yet don't have a visible polypide and which are enlarged in Figure 10. Circle Y zoid is enlarged in Figure 14. Drawn to Scale Z. (S) Stolons.

FIGURE 4. Another young colony. The parts are labelled for comparison with Figure 5: (Q) basal serrations; (P) polypide; (R) gut; (T) tentacles; (V) squared peristome rim; (W) proximal extension of Zoid U and classed as one of its "stolons" (S). A transverse septum is absent from it for a considerable distance. Stolon J was damaged along the dotted area. Letters G, H, J, L, M, N all represent particular stolons in which changes occurred in the two days which elapsed between conditions depicted in Figures 4 and 5. Individuals I, K, O and U show a developmental sequence. The youngest (I) is a sac without a polypide (P). Drawn to Scale Z.

PLATE II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confined to the edge of the young basal enlargement and were not present over its upper surface nor along the upright cylinder, consequently differing in spination from *Arachnidium fibrosum*, *Cylindroecium* (Nolella) *spinifera*, *C. horridum* (*N. horrida*) and *Nolella sawayai*.

Upright columnar zoid

The peristome rises upward from the basal enlargement, lengthening into a tall cylinder in time (Fig. 15). In retracted *Nolella blakei* zoids the peristomeal orifice is squared for a short distance (Figs. 12, 15, 16). In partly or fully extended zoids this character is not particularly noticeable (Figs. 7, 8, 11, 14, 18). This condition seems to obtain for *N. papuensis* also. In *N. annectens* the squaring appears to be of greater extent along the peristome than in *N. blakei*. The squared orifice distinguishes the genus Arachnoidea from Arachnidium. Arachnoidea has it while Arachnidium has a rounded peristomeal orifice. The main differences between *Nolella blakei* and the Arachnoidea species are in its taller zoids and smaller tentacle number (see Table II).

As *Nolella blakei* matures its zoids lengthen greatly vertically until they resemble slender, soft-walled columns. They are flexible and can twist about slightly as Figures 11, 14, 15, 17 and 18 show.

Polypide

The polypide consists of the tentacular crown (8 to 12 tentacles), the digestive tract and associated musculature.

The digestive tract terminology is in a nice state of confusion. Dr. Silén (1944) attempted to bring some order out of the chaos.

The digestive tract of *Nolella blakei* consists of the mouth, pharynx, esophagus, proventriculus (or gizzard?), stomach, intestine, rectum, and anus. The pharynx and esophagus are gray in color. The esophagus is exceedingly long in tall zoids (Figs. 15, 18). Whether the next part of the gut is a proventriculus or a gizzard could not be stated with certainty in the present material. In young zoids it was the same yellow color as the stomach. In other zoids it was yellower and thicker (a band). In still older zoids it seemed to have faintly defined teeth. Harmer and

---

PLATE II

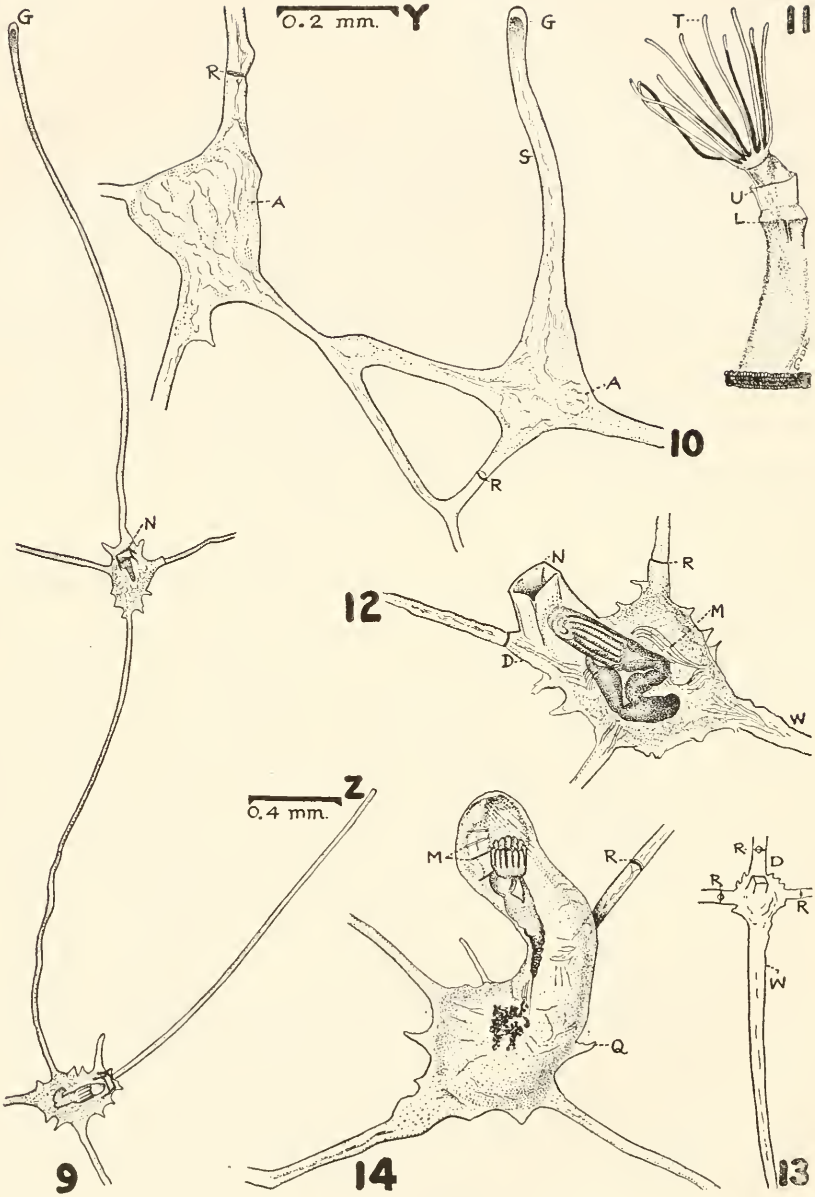
FIGURE 5. The colony of Figure 4 but drawn two days later, showing in solid black the growth and anastomoses of stolons (H, J, L, M, S, X) during that interval. The zoids (I, K, O) also have grown. Stolon N has changed its connections and the dotted part has degenerated. Drawn to Scale Y.

FIGURE 6. Diagram of polypide parts: (A) anus; (B) rectum; (C) intestine; (E) esophagus; (F) stomach or caecum; (P) gizzard? or proventriculus? and (T) tentacles.

FIGURE 7. A young zoid with 12 tentacles (T). Others in the same colony had 11. Other labels are: (D) distal part of zoid; (R) septa at the origin of the stolons; (S) stolon; (U) collar; (W) proximal extension of the zoid, one of the so-called stolons. Four stolons lead into the serrate base. Drawn to Scale Z.

FIGURE 8. A retracted zoid with 5 stolons at its base. The two distal (D) stolons anastomose. A clump of debris (V) obscures peristome area. Septa separate all stolons except the proximal one (W) from the expanded zoid base. Other labels are: (B) rectum; (C) intestine; (E) esophagus; (F) stomach; (Q) basal serrations. Drawn to Scale Z.

Plate III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13

others state however that a true gizzard is lacking in this genus. The caecum or stomach is typical of various bryozoa. The intestine is narrow. The rectum is narrow and very long in tall zoids. When the polypide retracts, the gut is withdrawn into the lower part of the body cavity in a rather twisted or folded fashion (Figs. 8, 15). When the polypide begins to emerge from the vestibule the short thin membranous collar (Fig. 18U) precedes the tentacular crown. Upon emergence and expansion of tentacles the collar is some distance below the tentacles (Fig. 7U). It is so transparent that it is easily overlooked. With the extrusion of the tentacular crown the pull on the gut is such that it straightens out the coils and twists of the tract.

Discussion

Nolella blakei appears to be an intermediate form between the Arachnidium, Arachnoidea, and Nolella genera. Its youngest zoids resemble the first two genera. Its mature zoids are definitely Nolella. The young *Nolella blakei* colonies resemble *Arachnoidea evelinae, A. protecta* and *A. ray-lankesteri* closely in the following respects. All four species have similar "stolons," squared peristome, and serrate basal enlargement, but *Nolella blakei* has fewer tentacles, and its short peristome elongates eventually into a long vertical zoid whose basal crenulations disappear with age. Other (minor) differences between *Nolella blakei* and the Arachnoidea species concern the vertical or linear extent of the peristomeal squaring and the proportionate size and diameter of peristome as compared to the basal enlargement. Also, *Nolella blakei* differs from *A. protecta* (Harmer, 1915, Plate III, Figs. 9, 10) in the much larger size of the latter's setigerous collar.

Nolella blakei resembles *Arachnidium fibrosum* (Marcus, 1938, Plate XII, Fig. 29A) in type of stolons and crenulated basal border. It differs from *A. fibrosum* in smaller tentacle number and in the absence of numerous bristle-like encrusted cuticular outgrowths originating from the peristome and basal enlargement's upper surface. Moreover, Arachnidium has a rounded peristomeal orifice rather than a squared one.

Plate III

Figure 9. An especially long young branch showing a transparent growing tip (G) and two young zoids with squared peristome (N). All septa have not yet formed. Drawn to Scale Z.

Figure 10. Eight stolons (S) and two zoid anlagen (A). This represents Circle X of Figure 3. Drawn to Scale Y.

Figure 11. Upper part of a zoid with extended polypide, showing 11 tentacles (T), transparent membranous collar (U) and a slightly debris-covered peristome (L). Drawn to Scale Y.

Figure 12. Young zoid with squared peristomeal orifice (N) and retracted polypide. Other structures: (D) distal part of zoid; (M) muscles; (R) septum and (W) proximal extension of zoid. Drawn to Scale Y.

Figure 13. Diagram showing relations of stolons and septa (R), with respect to the distal (D) and proximal (W) parts of a zoid, the flattened crenulated base and the rising squarish peristome in a very young colony. Considerable modification occurs in older zoids (see Figs. 15, 17, 18).

Figure 14. Detail of Circle Y of Fig. 3, shows a young flexible zoid beginning its upward growth. Drawn to Scale Y.

PLATE IV

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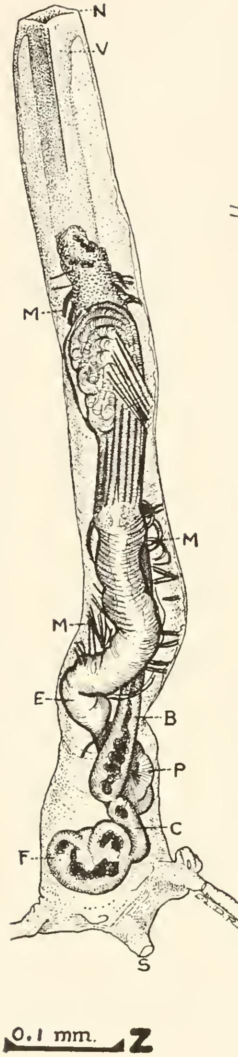
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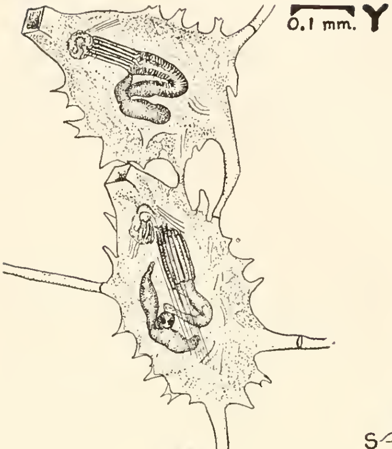

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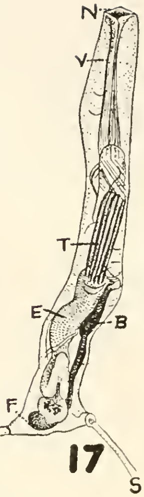

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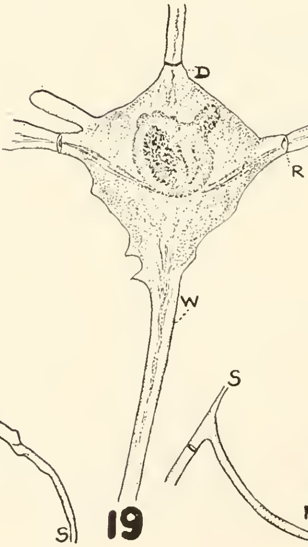

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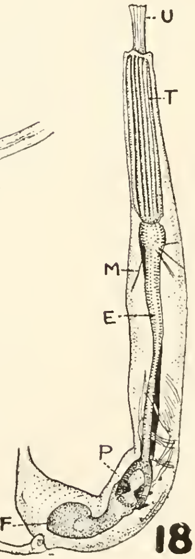

18

The genus Nolella (formerly Cylindroecium) contains a number of species, most of which differ from *Nolella blakei* in tentacle number (see Table II). *Nolella alta* and *Cylindroecium pusillum* both have about 10 tentacles. However, *Nolella alta* differs from *Nolella blakei* in having wider and longer zoids. These are about double or more the width and in some instances ten times as long as those of *Nolella blakei*. *Cylindroecium pusillum* (Hincks, 1880a, pages 537 to 538) differs slightly in appearance of the expanding "stolon" as it approaches the zoid base, being somewhat more like a small Victorella in that respect than is *Nolella blakei*. Hincks gives very little data on it. *Nolella blakei* differs from *Nolella sawayai* (Marcus, 1938, Plate XII, Fig. 30) in bodily proportions and cuticular outgrowths. Erect sawayai zoids are about as long but about twice as wide as those of blakei. Also, encrusted cuticular processes jut out in all directions from the upright tube and peristome in sawayai but not in blakei.

O'Donoghue incompletely described and figured three Nolella (Cylindroecium) species: *C. repens* (1923, page 50); *C. spinifera* (1924, Plate IV, Fig. 27, page 59) and *C. horridum* (1926, p. 61), but gave no measurements or tentacle numbers. His repens had basal processes but the zoids tapered too sharply from base to tip, like a wedge or sugar beet. His spinifera and horridum had numerous spines about the lower part of the peristome, so were quite unlike *Nolella blakei*.

In summary, *Nolella blakei* resembles in one way or another a number of Nolella, Arachnoidea and Arachnidium species but differs from most in tentacular number and from some species in other characteristics as growth habit, body proportions, relative size, etc.

SUMMARY

Perophora viridis, collected from Martha's Vineyard, Mass., and brought into the laboratory for culturing, yielded a bryozoan, *Nolella blakei* n. sp., which was cultured and observed alive for a time. *Nolella blakei* is an intermediate form whose young zoids resemble those of Arachnoidea and Arachnidium but whose older zoids are definitely Nolella. It was erected a new species on the basis of its tentacle number (8 to 12) and general zoarial and zooecial characteristics. It increases to 88 the number of bryozoans known from the general Woods Hole region.

PLATE IV

FIGURE 15. An older zoid, probably not full grown, shows the changed proportions of base to upright part of zoid. The digestive tract and zooecial tube are greatly elongated. The basal crenulations are absent. Labels: (B) rectum; (C) intestine; (E) esophagus; (F) stomach; (M) muscles; (N) squared peristomeal orifice; (P) gizzard? or proventriculus?; (S) stolon; (V) vestibule. Drawn to Scale Z.

FIGURE 16. An enlarged view of the two zoids of Figure 4 (P) as they looked after seven days' growth. The stolons between them are short. Drawn to Scale Y.

FIGURE 17. An older retracted zoid with a long gut. Labelled as in Figure 15, including tentacles (T). Drawn to Scale Y.

FIGURE 18. Another older zoid showing a stolon (S), the exceptionally long and narrow esophagus (E) and rectum, the partial extrusion of the collar (U) and the tentacles (T). Drawn to Scale Y.

FIGURE 19. A very young basal enlargement showing the anlage of the future polypide in the center. It was debris-covered and hence difficult to study. Labels: (D) distal part; (R) septum; (W) proximal extension of zoid. Drawn to Scale Y.

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X-RADIATION OF EGGS OF RANA PIPIENS AT VARIOUS MATURATION STAGES[1]

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INTRODUCTION

Interference with the normal course of embryological development has been the basis for many studies in experimental embryology. One of the most useful methods toward this end has been the study of abnormalities produced by various types of irradiation.

Many earlier investigators (Bohn, 1903; McGregor, 1908; Bardeen, 1909; O. Hertwig, 1911; G. Hertwig, 1911, 1912; P. Hertwig, 1916), working with various types of gametes and embryos, attempted to ascertain the effects produced by treatment with x-rays and radium. As stated by Butler (1936), however, "The work which has been done in this field has been, for the most part, qualitative in nature, and in many cases, particularly in the earlier investigations, the qualitative results are not thoroughly trustworthy. In many cases the amount of radiation which reached the egg or embryo was either unknown or at least unstated, the area of the embryo which came under the influence of the radiation was undetermined and little attention was given to the influence of external factors other than radiation, such as change of temperature or chemical changes in the medium." Since the standardization of the roentgen unit at the Fifth International Congress of Radiation in 1937, work in the field has been aimed at a more controlled study of the effects of accurately controlled doses of irradiation on adequate numbers of the various embryological stages.

The purpose of the present investigation was to make a study of the effects of x-radiation on the eggs of *Rana pipiens*. Since, in *Rana pipiens*, the ovarian eggs possess germinal vesicles and the uterine eggs are in the metaphase of the second maturation division, it was possible to study the effects of the x-radiation at two different maturation phases. A comparison was also made with eggs irradiated shortly after fertilization when the second polar body is being formed. It was thought, too, that a comparison of x-radiation studies on the eggs of *Rana pipiens* with the x-radiation studies of Rugh (1939) on the sperm of the same species would prove of value.

I wish to express my gratitude to Professor Roberts Rugh[2] for suggesting this problem and for his guidance through the course of the work, and to Dr. Titus C. Evans of the Radiological Research Laboratory, Columbia University, for so graciously making available the x-ray facilities.

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[1] A dissertation in the Department of Biology submitted to the Faculty of the Graduate School of Arts and Science of New York University in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

[2] Present address: Radiological Research Laboratory, College of Physicians and Surgeons, Columbia University, New York, N. Y.

MATERIALS AND METHODS

Rana pipiens were obtained during their period of hibernation, from October to May, from Alburg, Vermont, and were kept on a water table in running water in the laboratory. Eggs were obtained by the injection of pituitary glands and fertilized according to the method of Rugh (1948).

Irradiation was carried out by means of two Coolidge-type water-cooled tubes at 180 kv. peak and 30 milliamps. Filters consisting of two mm. of copper plus one mm. of aluminum were used for all except the highest dose of 41,600 r. which was unfiltered. The intensity for the doses ranging from 100 r. through 1600 r. was 50 r./min. at a target distance of 36 cm.; that for doses ranging from 3200 r. through 25,600 r. was 675 r./min. at a target distance of 9.8 cm.; that for doses of 41,600 r. was 4150 r./min. at a target distance of 9.8 cm. The temperature ranged from 26° C. to 27° C.

It was necessary that the method of administering the irradiation vary slightly for the eggs at different stages of maturation.

(1) The ovarian eggs were treated by x-raying the female donor in the region of the ovaries. The females were then injected with pituitary glands and forty-eight hours later the eggs were stripped into a normal (unirradiated) sperm suspension. As soon as the jelly coating swelled, the eggs were separated into groups of from three to five. Following first division of the zygote, cleaved eggs were separated from uncleaved, and the eggs which had cleaved were placed in tanks measuring 24 in. × 12 in. × 6 in. in four inches of tap water at 67° to 70° F. in which constant aeration was maintained. Lettuce and Elodea were supplied as food material after the animals had hatched. Eggs from unirradiated females handled in the same way were used as controls.

(2) For the study of uterine eggs, female frogs were injected with pituitary glands forty-eight hours prior to irradiation to bring the eggs down into the uteri. After the removal of a few eggs into normal sperm suspension as controls, the females were irradiated in the region of the uterus. In this group it was possible to strip some eggs into sperm suspension after the administration of each irradiation dose so that one female could be used to provide eggs for all dosage levels. The same procedure was then followed as has been described for the ovarian eggs.

(3) A few studies of the effect of x-radiation on fertilized eggs were made with unfiltered radiation from a water-cooled Coolide-type tube at 185 kv. peak and 25 milliamps.[3] In these studies, normal eggs were stripped into dishes of normal sperm suspension and the dishes of eggs were irradiated with doses from 33 r. to 1000 r. approximately twenty minutes after insemination (at the time when the second polar body was being formed). After irradiation, the eggs were handled as described above.

Counts were made of cleaved eggs, gastrulae, neurulae, hatched tadpoles and normal tadpoles at each dosage level. Counts of gastrulae included all those individuals that showed a dorsal lip of the blastopore. Counts of neurulae included all those individuals that showed elongation and evidence of neural folds.

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[3] The x-ray facilities for this part of the work were made available through the kindness of Dr. R. S. Anderson of Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York.

The percentages for each stage were calculated on the basis of the preceding stage in order to eliminate the possibility that the percentages for each of the various stages would reflect the effects of the irradiation on the previous stage.

OBSERVATIONS

Jelly of irradiated ovarian and uterine eggs

Damage to the jelly surrounding the eggs, such as has been reported for the jelly of the eggs of Arbacia (Evans, Beams and Smith, 1941) following irradiation was not observed except on the ovarian eggs subjected to the highest doses (25,600 r. and 41,600 r.). The jelly on the eggs from some of the animals receiving these high doses was opaque, flaccid and watery. Animals producing such eggs were found upon autopsy to have very shrunken oviducts and hemorrhagic uteri much distended with jelly. Because of this fact, and the fact that there was no noticeable effect on the jelly of the uterine eggs which were irradiated after they had traversed the oviduct, it is thought possible that the effect noted on the jelly of the ovarian eggs was an indirect one, caused by irradiation damage to the oviducts.

Cleavage of irradiated ovarian and uterine eggs

The cleavage rate and pattern were the same in the irradiated as in the control eggs, although a considerably higher percentage of ovarian eggs than uterine eggs cleaved at each x-ray dose as can be seen from Table 1 and Figure 1. In each of the two groups, the percentage of cleavage drops off with increasing irradiation (though more gradually in the ovarian curve), rises slightly, and again drops until at 41,600 r. only 3.4 per cent of the ovarian eggs cleave and none of the uterine eggs cleave. Thus these curves show a slight "paradoxical effect" (Dalcq, 1930) or an inverse relationship between the radiation dose and the damage produced.

TABLE 1

Cleavage

X-ray dose in roentgen units	Ovarian eggs			Uterine eggs		
	No. eggs	No. cleaved	%	No. eggs	No. cleaved	%
Control	780	750	96.15	526	469	89.16
100 r.	400	392	98.00	346	292	84.39
200 r.	936	909	97.11	500	386	77.20
400 r.	409	400	97.80	696	492	70.70
800 r.	498	352	70.70	742	385	51.88
1,600 r.	1,190	815	68.48	796	394	49.49
3,200 r.	1,043	912	87.44	742	393	52.96
6,400 r.	1,036	811	78.28	696	454	65.22
12,800 r.	523	400	76.50	785	361	45.98
25,600 r.	2,321	1,408	60.66	833	96	11.52
41,600 r.	1,661	56	3.37	500	0	0

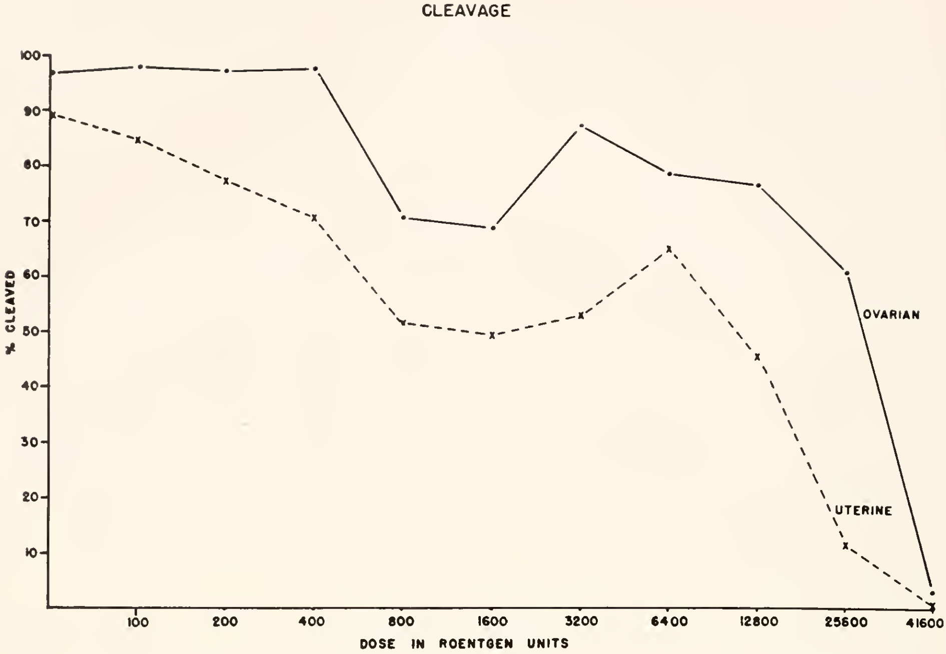


FIGURE 1.

Gastrulation of irradiated ovarian and uterine eggs

It appears from Tables 2 and 3 and Figures 2 and 3 that irradiation with x-rays even at high doses does not affect appreciably the ability of either the ovarian or uterine eggs to undergo morphogenetic movements at least up to the formation of a dorsal lip. The number of abnormal gastrulae, however, markedly increases with

TABLE 2

Ovarian eggs

(Each percentage based on previous stage)

X-ray dose in roentgen units	No. eggs cleaved	Per cent Gastrulated	Per cent Neurulated	Per cent Hatched	Per cent Normal
Control	780	99.20	99.19	100	99.59
100 r.	400	99.25	100	99.75	97.73
200 r.	1,600	98.25	98.22	90.28	87.73
400 r.	800	99.75	100	99.00	92.41
800 r.	3,669	97.66	96.60	96.62	48.53
1,600 r.	782	100	98.72	93.99	26.09
3,200 r.	1,600	97.00	90.59	70.27	28.34
6,400 r.	1,182	97.12	81.45	60.43	30.27
12,800 r.	520	96.92	95.63	64.94	16.61
25,600 r.	935	94.33	78.23	46.23	19.12
41,600 r.	56	87.50	71.43	17.14	0

TABLE 3

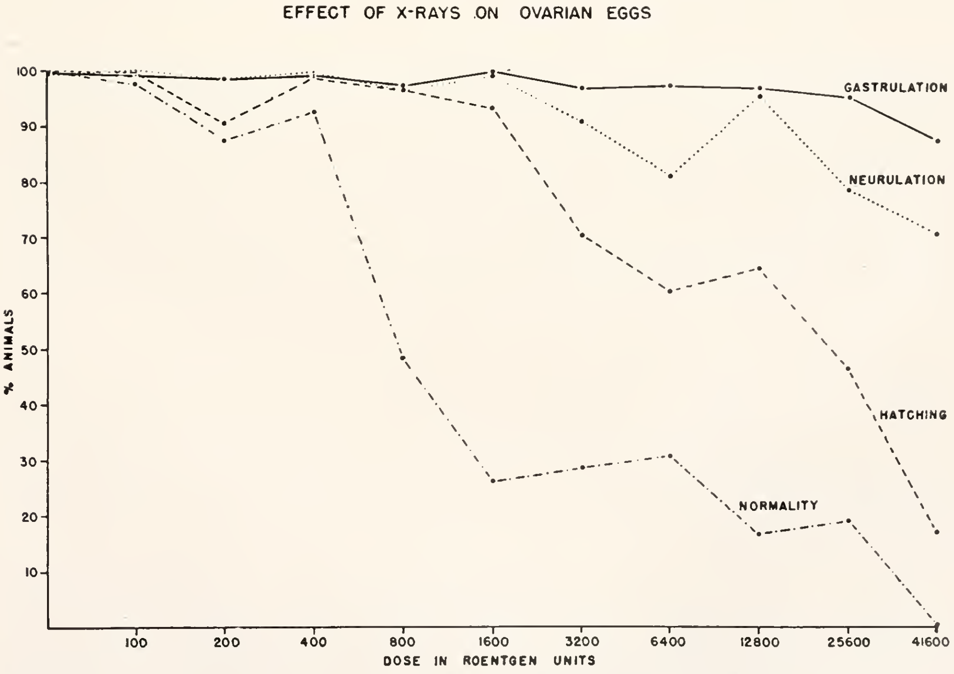
Uterine eggs

(Each percentage based on previous stage)

X-ray dose in roentgen units	No. eggs cleaved	Per cent Gastrulated	Per cent Neurulated	Per cent Hatched	Per cent Normal
Control	825	98.66	97.30	97.10	97.79
100 r.	554	97.47	97.59	97.15	80.47
200 r.	606	98.18	98.82	84.69	49.80
400 r.	860	98.48	94.21	76.44	10.98
800 r.	676	97.92	93.35	50.97	0.32
1,600 r.	612	91.66	90.73	49.12	5.60
3,200 r.	791	98.86	91.18	63.81	5.49
6,400 r.	758	97.09	90.48	64.26	4.21
12,800 r.	600	94.83	90.69	64.53	2.70
25,600 r.	198	94.94	77.66	46.58	4.62
41,600 r.	0	0	0	0	0

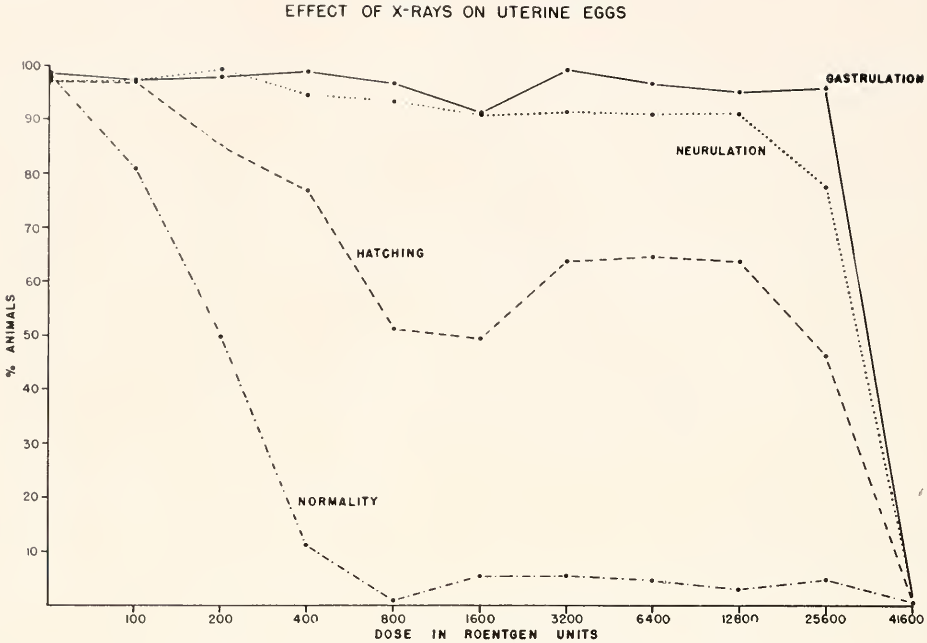
increased irradiation. Since at a dosage of 41,600 r. none of the uterine eggs cleaved, no further data could be obtained on these eggs at this dosage.

The types of abnormal gastrulae produced are very similar in all groups. The severity of the abnormalities, however, increases with increasing dosage and is more



For each point on each curve, the percentage is based on the number of animals which reached the preceding stage of development

FIGURE 2.



For each point on each curve, the percentage is based on the number of animals which reached the preceding stage of development

FIGURE 3.

marked in most of the uterine batches than in the corresponding ovarian groups. There are those blastulae which gastrulate apparently normally through the formation of a dorsal lip and then cytolyze. Some form both dorsal and lateral lips, and some develop abnormally large yolk plugs of various sizes. In all of these groups, it seems evident that epiboly continues without the inturning of material, since many of the gastrulae have irregular, "warty" surfaces. Some exogastrulae are also found. Many of the animals which continue growing, but do not neurulate, take on extremely irregular and amorphous forms.

Neurulation of irradiated ovarian and uterine eggs

From Tables 2 and 3 and Figures 2 and 3 may be seen the percentages of those embryos which neurulated, based on the number that gastrulated. It is evident that a very large percentage of those animals which gastrulate also neurulate. Of the embryos that neurulated a fairly high percentage was normal in the ovarian group except at the two highest doses. In the uterine group, however, there were fewer normal neurulae.

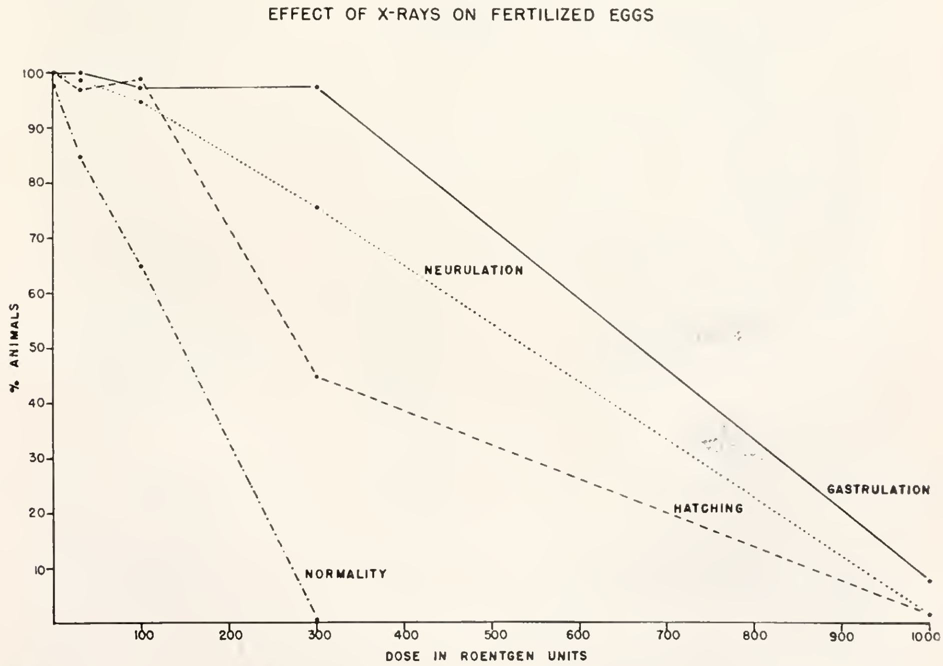
There are numerous types of abnormal neurulae found in the irradiated groups of animals. These range from those which go no further than slight elongation and flattening of the dorsal surface to those with slightly incomplete posterior closure of the folds because of the persistence of a yolk plug. Included in the group are those which form just the suggestion of short, low neural folds; those which form only one fold; those which form well-defined folds which do not close; and those which form folds which close only partially. Most of these abnormal forms have persistent yolk plugs.

Hatching of irradiated ovarian and uterine eggs

The number of animals hatching from irradiated ovarian eggs (Tables 2 and 3 and Figures 2 and 3) declines with increasing dosage while the hatching curve for uterine eggs shows some evidence of a "paradoxical effect". It should also be noted that, here again, the lower irradiation doses cause a more rapid initial drop in the uterine than in the ovarian curve.

Among the hatched tadpoles, there are various abnormal forms. Again, it is evident that the animals developing from eggs which were x-rayed in the metaphase of the second maturation division (eggs in the uterus) are more abnormal (Plates 3 and 4) than those developing from eggs x-rayed in the germinal vesicle stage (eggs in the ovary) (Plates 1 and 2). Surprisingly enough, a few of the animals developing from ovarian eggs which had been given a dose of 25,600 r. appear quite normal (Plate 2). Among the tadpoles from each group, however, are found some microcephalics; some with spina bifida; some stubby, curved, typically haploid appearing; some with short, crumpled, curled, notched or clipped tails; some with papillated surfaces; some edematous and some of quite amorphous shapes with large remaining yolk plugs or yolk masses exposed.

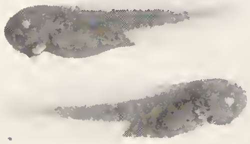
In Tables 2 and 3 and Figures 2 and 3 are seen the percentages of those animals which hatched which were normal in appearance. The ovarian curve again is


EFFECT OF X-RAYS ON FERTILIZED EGGS

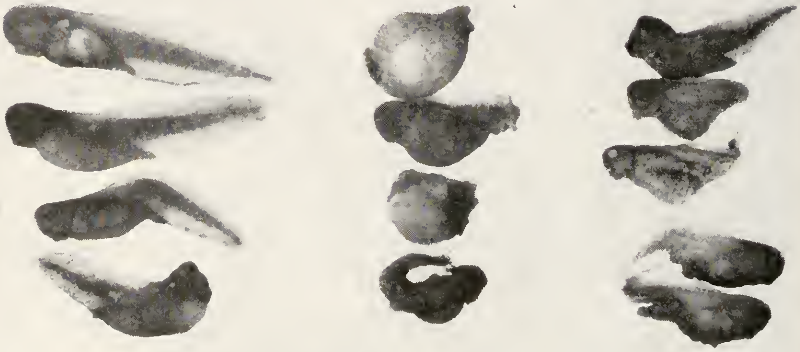
For each point on each curve, the percentage is based on the number of animals which reached the preceding stage of development

FIGURE 4.

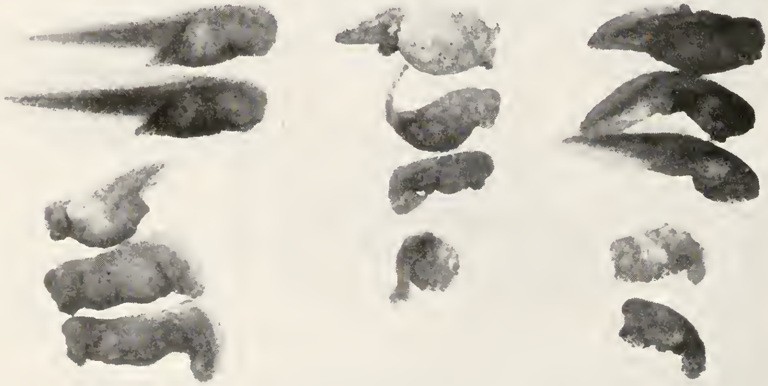
PLATE I



CONTROLS

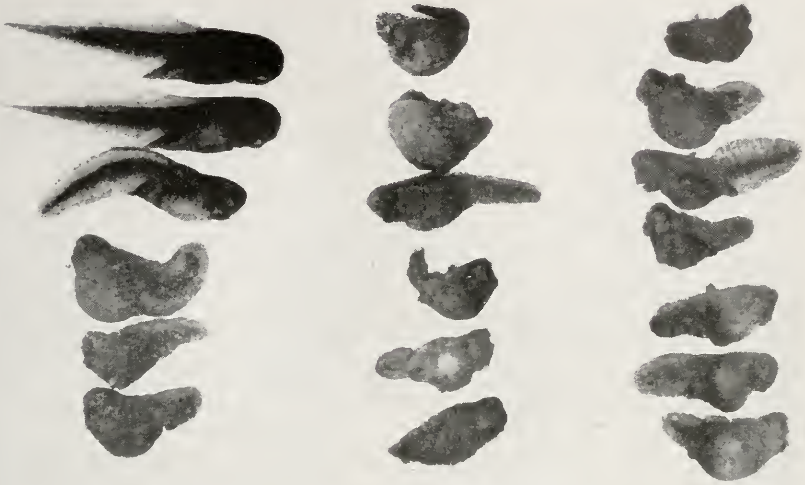


OVARIAN EGGS EXPOSED TO 400 ROENTGEN UNITS

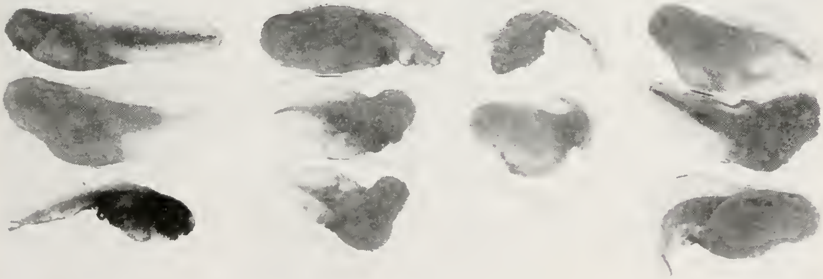


OVARIAN EGGS EXPOSED TO 1600 ROENTGEN UNITS

PLATE 2



OVARIAN EGGS EXPOSED TO 6400 ROENTGEN UNITS

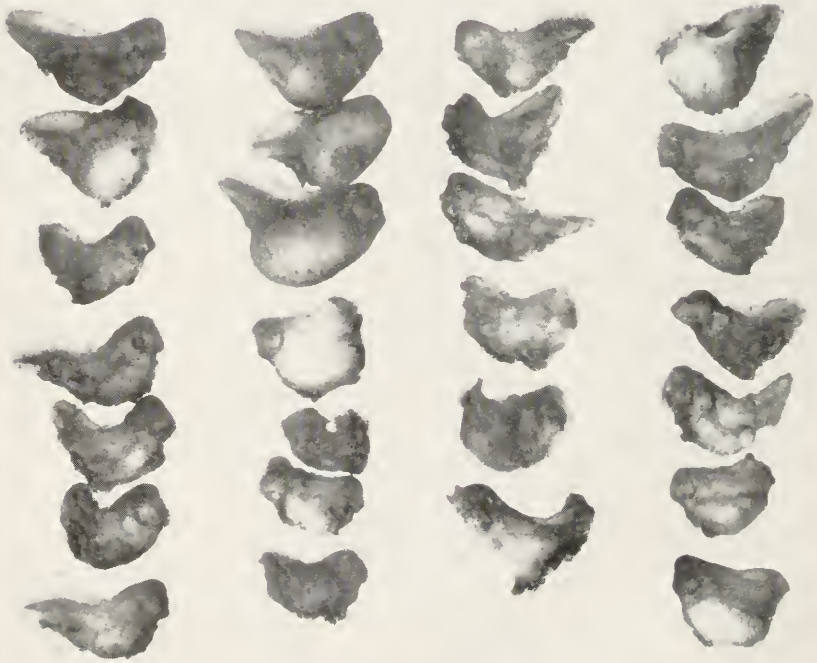


OVARIAN EGGS EXPOSED TO 25600 ROENTGEN UNITS

OVARIAN EGGS EXPOSED TO 41600 ROENTGEN UNITS

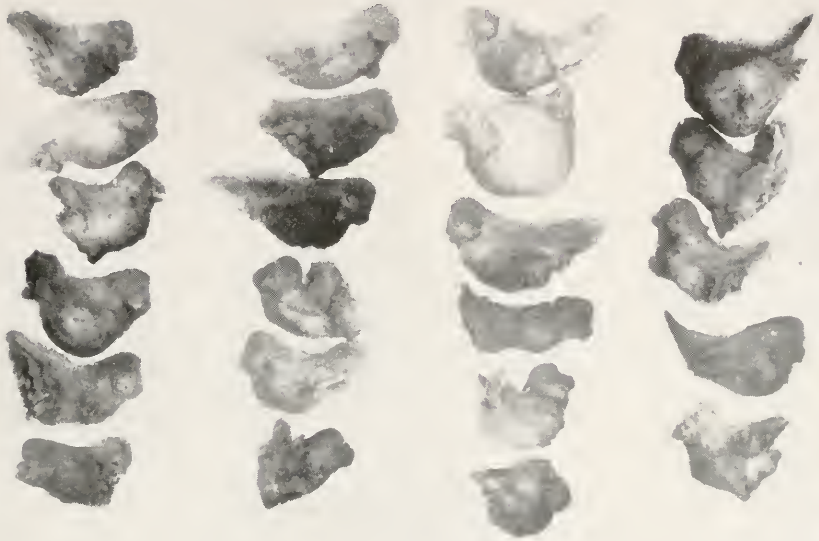
PLATE 3

UTERINE EGGS EXPOSED TO 400 ROENTGEN UNITS

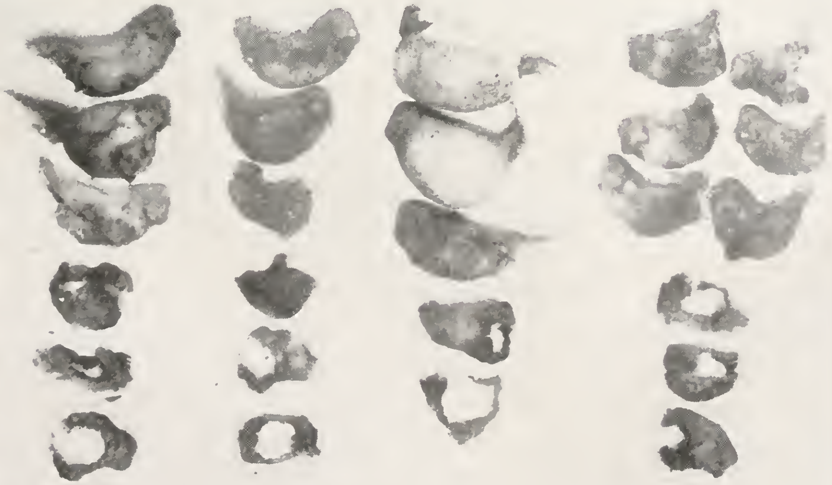


UTERINE EGGS EXPOSED TO 1600 ROENTGEN UNITS

PLATE 4



UTERINE EGGS EXPOSED TO 6400 ROENTGEN UNITS



UTERINE EGGS EXPOSED TO 25600 ROENTGEN UNITS higher than the uterine curve. There seems to be a gradual effect on the ovarian eggs at dosages of 100 r. through 400 r. This is followed by a rapid drop to 1600 r. and then a very gradual decline. The effect on the uterine eggs is, however, somewhat different. Here there is a very rapid drop to 800 r. followed by an extended very low plateau.

Thus it may be seen that the effect of the irradiation has, in general, been less on the ovarian eggs.

Irradiation of fertilized eggs

The results of irradiating fertilized eggs which were in the process of giving off their second polar bodies may be seen in Table 4 and Figure 4. It is evident that a dose of 100 r. allows most of the eggs to gastrulate, neurulate, and hatch. At this dosage approximately 65 per cent of the resulting tadpoles are normal in appearance. However, at 300 r. there is a marked drop in the number which neurulate and hatch, and none of the animals is normal. At a dosage of 1000 r. some of the eggs gastrulate, but none goes on in development. The types of abnormalities seen are much the same as those resulting from irradiation of the eggs alone.

TABLE 4

Fertilized eggs

(Each percentage based on previous stage)

X-ray dose in roentgen units	No. eggs cleaved	Per cent Gastrulated	Per cent Neurulated	Per cent Hatched	Per cent Normal
Control	200	100	100	100	97.50
33 r.	208	100	98.08	97.06	84.34
100 r.	190	97.37	94.59	98.86	64.74
300 r.	452	97.57	75.74	44.31	0
1,000 r.	250	9.60	1.43	0	0

Delayed fertilization

Since fertilization can be accomplished only after the eggs have traversed the oviduct, it was necessary, in the case of the ovarian eggs, to delay a minimum of twenty-four hours between irradiation and fertilization (i.e., the length of time necessary for stimulation by the pituitary glands to bring the eggs from the ovary to the uterus). Since recovery from irradiation has been reported (Packard, 1930; Henshaw, 1932; Evans, 1934, et al.), it was decided to test whether the lesser effect of the irradiation on the ovarian eggs was the result of recovery of the eggs during the period of delay rather than a lower susceptibility of the ovarian eggs. Therefore a few batches of uterine eggs were delayed for a period of forty-four hours between irradiation and fertilization. The results of such a test are indicated in Table 5. (Eggs from the same female were used for both batches.)

It is evident from this that the uterine eggs, at least, do not seem to recover from irradiation effects if a period of time is allowed to elapse between irradiation and fertilization. The general trend seems to be in the other direction, i.e., the delay results in slightly poorer development.

TABLE 5

Delayed fertilization

	Fertilization immediately after irradiation	Fertilization delayed 44 hours after irradiation
Per cent Gastrulated	99.18	96.42
Per cent Neurulated	95.90	83.92
Per cent Hatched	63.52	48.21
Per cent Normal	1.22	5.35

High intensity effects

For the sake of convenience, the irradiation intensity differed at the low and high doses, as was mentioned above. Thus the irradiation administered for doses 100 r. through 1600 r. was at the rate of 50 r./min. while that administered for doses 3200 r. through 25,600 r. was at 675 or 685 r./min.

To test whether this intensity difference was affecting the results, as has been reported by some other workers (Forssberg, 1933; Sax, 1939), some uterine eggs were given a total dose of 800 r. at the rate of 675 r./min. The results of that test compared with the average at the lower intensity at a total dose of 800 r. are shown in Table 6.

TABLE 6

The effect of different intensities of x-radiation

	800 r. at 50 r. per min.	800 r. at 675 r. per min.
Per cent Gastrulated	97.92	99.18
Per cent Neurulated	91.42	95.90
Per cent Hatched	46.60	63.52
Per cent Normal	1.48	1.22

It is evident from this test that irradiation at a higher intensity is not more damaging than that at a lower one. Thus the intensity factor does not seem to be involved in this work. The formula D = TI (Dose = time × intensity) seems to be valid for this work.

DISCUSSION

The significance of the foregoing results will be better understood if they are compared in the light of previous research.

A study of the sensitivity to x-radiation of the stages of maturation of the eggs of *Rana pipiens* has been made in the present work. Many attempts have been made in the past to test the difference in susceptibility to irradiation between resting cells and those in varying phases of meiosis or mitosis. Most of the results, from widely diverse groups of plants and animals, have pointed at least to the fact that a cell undergoing division is more sensitive than is a resting cell (Giese, 1947). It was possible in the present work to test resting cells (ovarian eggs), cells in metaphase of the second maturation division (uterine eggs) and those completing the second maturation division (fertilized eggs). The results show quite clearly that the cells undergoing maturation division are the more susceptible to damage by x-rays. The fertilized eggs were obviously the most sensitive, but the studies on these eggs were complicated by the necessity of irradiation of the sperm along with the eggs.

The differences in susceptibility between resting and dividing cells have been explained by Heilbrunn and Mazia (1936) on the basis of the greater rapidity with which calcium released from the cytoplasm by the irradiation can reach the chromosomes when no nuclear membrane is present. Sparrow (1944) has correlated sensitivity with nucleic acid metabolism; the most sensitive stages being those which have a high content of desoxyribose nucleic acid or nucleotides.

It should be noted here that throughout the course of this work considerable variation in susceptibility of the eggs of the frog to the effects of x-radiation has been observed. Similar findings have also been reported following irradiation of the eggs of Triton (P. Hertwig, 1916) and Chaetopterus (Packard, 1918) and following treatment of the eggs of the frog with agents such as 2, 4-dinitrophenol (Dawson, 1938) and colchicine (Keppel and Dawson, 1939). One factor that may possibly be involved is the varying genetic constitution that would be expected in any wild population as judged from the work done on Drosophila (Dobzhansky, 1939). It is also interesting to note that inherited differences in sensitivity to ultra-violet rays and x-radiations have been reported in a strain of bacteria (Witkin, 1946, 1947). This variation coupled with work on small numbers of animals and lack of a standardized x-ray unit may be the reason for so many conflicting reports in the field.

It was thought that a comparison of the effects of x-radiation of the eggs of *Rana pipiens* with studies on the sperm of the same species (Rugh, 1939) would prove of value. Such a comparison shows that the sperm are more sensitive than are the eggs. However, the so-called "paradoxical effect," an inverse relationship between the radiation dose and the damage produced, reported after exposure of various gametes to x-rays, ultra-violet and radium radiations (O. Hertwig, 1911; G. Hertwig, 1911; Simon, 1930; Dalcq, 1930; Rugh and Exner, 1940), is not nearly so marked in the present work on the eggs as it has been shown by Rugh (1939) to be on the sperm. The cause of the paradoxical effect has been claimed, from cytological studies (G. Hertwig, 1911), to be a gradual destruction of the irradiated chromosome complement with increased dosage until, at the higher doses, development is haploid and is carried on under the sole influence of the normal chromosome complement. Dalcq (1929), however, is of the opinion that the paradoxical effect is only explicable in all its details by the intervention of the nucleoplasm. Probably a true paradoxical effect on the eggs is prevented by irradiation damage to the cytoplasm, and in the case of the ovarian eggs to a prevention of maturation of those eggs which are very badly damaged. That cytoplasmic damage does occur and does affect the nucleus has been shown by the work of Duryee (1939 a and b, 1947). It is obvious that no paradoxical effect would be possible in the eggs irradiated when the second polar body was being formed and that the x-radiation effects on these eggs would be much more severe, since in these studies both the egg and sperm have been irradiated.

The last part of the problem to be discussed is the effect of the irradiation on the mechanics of development. The lack of effect on cleavage rate and pattern found in the present work agrees with the results of Ancel and Vintemberger (1925) on Rana eggs in the two-cell stage, Dalcq (1929) on unfertilized Rana eggs and Rugh (1939) on *Rana pipiens* sperm. However, accelerated cleavage has been reported for fertilized amphibian eggs by other workers (Gilman and Baetjer, 1904;

Hoffman, 1922). Lack of controlled temperature may have been a factor in the latter work. There is some indication, too, that fertilized eggs subjected to very much higher doses of x-ray than were used in the present work will delay cleavage and alter the cleavage pattern (Langendorff and Langendorff, 1933).

Cleavage appears to be a critical stage for the x-ray damaged eggs to accomplish. It would seem that if the eggs cleave, at least some morphogenetic movements will take place in almost all of them. As has been mentioned, however, there is a fairly marked drop in the numbers of normal gastrulae.

A large percentage of those embryos which show any gastrular movements will also at least attempt neurulation, the amount of neurulation being dependent on how far gastrulation has progressed. The number of normal neurulae corresponds quite closely with the number of normal gastrulae. This seems to be a good indication that the normality of the neurulae is directly dependent on the normality of the gastrulae.

Not all the animals that appear to have undergone normal neurulation movement are able to hatch. This may be an indication of lack of a hatching enzyme or other fundamental cellular disarrangements. The number of normal tadpoles produced is obviously very much lower than the numbers of normal gastrulae or neurulae. It would thus almost appear that there are two major effects of the x-radiation; one immediate effect which inhibits cleavage and the other which becomes most apparent at the time of hatching. If the eggs cleave, a fairly high percentage continues development to become normal gastrulae and neurulae, and an even higher percentage shows at least abnormal gastrulation and neurulation movements. The second effect appears after neurulation in the producation of a high percentage of abnormal tadpoles. Some of these are the further development of abnormal neurulae, but many of them appear to develop from normal-appearing neurulae.

The abnormalities resulting from x-radiation of the eggs of *Rana pipiens* seem similar to those obtained by Rugh (1939) in x-radiation of the sperm of the same form. Similar abnormalities have also been obtained in amphibians by subjecting developing embryos to supra-maximal temperatures (Hoadley, 1938), to 2, 4-dinitrophenol (Dawson, 1938), by fertilizing over-ripe eggs (Witschi, 1930 and Zimmerman and Rugh, 1940), by placing fertilized eggs in colchicine solutions (Keppel and Dawson, 1939) and by subjecting fertilized eggs to hydrostatic pressure (Rugh and Marsland, 1943). Any of these agents may in some way (1) affect the surface tension of the cells and or the pH of the blastocoelic fluid to prevent normal blastoporal invagination and the infolding of the neural plate (Holtfreter, 1943), (2) either completely or partially damage the ectoderm to prevent its normal spreading (Holtfreter, 1943) and or (3) damage the endoderm to prevent its spreading and impair the polarity of the mesodermal cells (Holtfreter, 1944).

Although most of the agents, other than x-rays, probably damage the nucleus indirectly by first damaging the cytoplasm, while x-rays probably damage both directly (Failla, 1937), the abnormalities ultimately produced are similar.

Summary and Conclusions

1. Eggs of *Rana pipiens* in germinal vesicle stage (ovarian eggs), metaphase of the second maturation division (uterine eggs) and during completion of the second

maturation division (eggs twenty minutes after fertilization) were irradiated with doses of roentgen units ranging from 100 r. to 41,600 r. Counts were made of cleaved eggs, gastrulae, neurulae, hatched embryos and normal tadpoles (i.e., embryos beyond stage 25).

2. The jelly surrounding the eggs was apparently unaffected except on the ovarian eggs at the highest irradiation doses (25,600 r. and 41,600 r.). Damage at these doses may have been an indirect effect of the x-rays on the oviduct.

3. The cleavage rate and pattern were not affected. The percentage of cleavage was very much lowered, however, by the irradiation.

4. The lower doses of x-rays had relatively little effect on the ovarian eggs. At a dose of 400 r., for instance, 97.8 per cent cleaved, 99.75 per cent gastrulated, 99.75 per cent neurulated, 98.75 per cent hatched and 91.25 per cent appeared normal.

5. The uterine eggs were more sensitive to x-radiation than were the ovarian eggs, while the fertilized eggs were by far the most sensitive of the three groups tested. Thus, when a dose of 1000 r. was administered to the fertilized eggs, only 9.6 per cent of the animals gastrulated, 1.4 per cent of these showed evidence of neurulation and none hatched. A dose of 41,600 r. inactivated the uterine eggs, while at this latter dosage, although only a small percentage of the ovarian eggs cleaved, of those that cleaved, 87.5 per cent gastrulated, 62.5 per cent neurulated, and 10.71 per cent hatched.

6. The types of abnormalities produced by irradiation of the eggs are similar to those resulting from irradiation of the sperm of the same species.

7. A delay of twenty-four hours between irradiation and fertilization of the uterine eggs, to simulate the required delay for the ovarian eggs, appeared to have no effect. Thus there seems to be no recovery from the effects of the x-radiation.

8. There is no difference in the effectiveness of the different x-ray intensities used in this work as long as the total roentgen exposure is the same.

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STATISTICAL AND PHYSIOLOGICAL STUDIES ON THE INTERPHASIC GROWTH OF THE NUCLEUS

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1. *Introduction.* The rhythmic growth of the nuclear size.
2. *The study of the nuclear growth.*
    - (a) Statistical methods.
    - (b) Physiological methods.
    - (c) The caryometric analysis of spermatogenesis.
    - (d) The caryometric analysis of uterine tissues during the sexual cycle.
    - (e) The caryometric analysis of the ovarian endocrine tissues.
3. *Discussion and conclusions.*
4. *Summary.*
5. *Literature cited.*

1. INTRODUCTION

1. (*a*) *The "rhythmic" growth of nuclear size*

We define as "rhythmic growth" that growth of the cell nucleus in which periods of quick development alternate with others of rest or slower growth. The volumes attained in each step of this growth are generally in a simple mathematical ratio.

The simplest rhythmic growth is that in which nuclear size is doubled at every step (Jacobj, 1926). By measuring the nuclear sizes (volumes) of a homogeneous tissue, such as, for instance, the mammalian liver, and distributing the values into classes of a frequency curve ("Statistiche-kariometrische Untersuchungen), Jacobj verified that there are many modal classes, and that these modes form a regular series with a ratio of 1 : 2.

Some studies following the discovery of Jacobj led to the explanation that rhythmic growth of the nucleus is due to the reduplication of the nuclear .content, which establishes in the tissues a series of polyploid nuclei or nuclei with polytene chromosomes (D'Ancona, 1939–40–41 ; Biesele, Poyner and Painter, 1942). Biesele later published a series of papers dealing with the size of the chromosomes in different conditions, ages, and tissues. We cannot discuss these results here because they do not appear to be sufficiently defined in relation to nuclear volume and nuclear growth.

In the meantime, other studies revealed that the steps in rhythmic growth of the nucleus may occur at a ratio different from that of 1 : 2. To some authors this fact seems difficult to interpret in genetic terms (Wermel and co-workers). Several authors described a rhythmic growth with a ratio of 1 : 1, 5 (Wermel and Portugalow, 1935; Bogojawlensky, 1935), and others found the ratio to be 1 : 1.41, which is the value of the square root of 2 (Brummelkamp, 1939 and G. Hertwig, 1938–39). The nature of these intermediate stages, which German-speaking authors call "Zwischenklassen," has been discussed from different points of view in a previous paper (Schreiber, 1943). Both Brummelkamp (1939) and Hertwig (1938–39) studied the problem from a physiological point of view without considering the genetic basis of the phenomena that form nuclear activity during the interphase.

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The main object of the present paper is the study of the intermediate classes of nuclear size during the reduplication step.

For this purpose, statistical analysis of nuclear size has been used, as by previous authors, in actively reproducing tissues as well as contemporary physiological methods such as hormone stimulation. With this method we can arrest or increase the mitotic activity of the cells by specific stimulation, and thus define the limits of the interphasic variation of nuclear size.

For the statistical study of nuclear growth some basic facts must be ascertained. First we must find out whether there is any correlation between nuclear volume and multiple value of the genome,[2] and if so at what point in the nuclear cycle it occurs.

The problem as to whether the number of chromosomes is related to the volume or to the surface of the nucleus, or to some other quantitative value of the cell is still open to discussion.

The second fact to be established is the limit of the variation of nuclear size during an interphasic growth cycle, with reference to the volume of a nucleus with a genome of known quantitative value. The term "interphase" is here used to indicate in a general way the period between two somatic divisions. Although the term was originally used to signify the interval between the first and second meiotic divisions, it was subsequently used by various authors as a synonym for "resting" or "metabolic" stage.

Another important fact which must be established in these caryometric studies is whether modifications of the model value of nuclear size in a tissue are natural or experimentally induced. We must be sure that variations in mode represent true growth or reduction and are not due to the occurrence of various types of cells of different characteristic sizes (i.e. grade of ploidy) originally present in the tissue and assuming by differential proliferation, the role of the main cell under the new conditions (Schreiber and Romano-Schreiber, 1941; Paccagnella, 1944–45).

In previous papers we approached these different problems from different angles and with different materials, each offering characteristics that would answer one of the questions. Thus the problem of the relationship between nuclear size and genome has been studied in a series of polyploid Coffea plants (Schreiber, 1946) and in the spermatogenesis of snakes (Schreiber, 1946–47). The problem of the nature of the steps in rhythmic growth, and the limits of the mitotic interphase has been studied in experiments on the uterine cells under different physiological conditions and on the granulosa layer and luteal cells of the mammalian ovary (Salvatore and Schreiber, 1947; Schreiber, Mello and Salvatore, 1948; Salvatore, 1948).

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[2] The term "genome" is here used following the definition of Sharp (Introduction to cytology, 3d Ed., New York, pg. 121): "In any given kind of plant or animal each nucleus contain an outfit or complement of chromosomes composed of a certain number of members showing characteristic difference in form and function. As a general rule the nucleus of an egg before fertilisation contains a complement made up of one each of several kind of chromosomes, such a complement is called a *set* or *genome*"; and pg. 353: "The monoploid chromosome set *or genome* is a group of chromosomes differing among themselves in the number and kind of their component elements. Ordinarly all or nearly all of the genetic elements (genes) are probably necessary to the normal activity of the nucleus; in other terms the genom is a harmonious differentiated system of elements, the majority of which are essential parts of the system."

We shall here summarize those studies which contribute to the knowledge of nuclear growth during interphase.

2. The Study of the Nuclear Growth

2. (*a*). *Statistical methods*

Analysis of the statistical distribution of nuclear sizes in a homogeneous mass of cells of some tissues verifies the existence of various modal values. This indicates that the nuclei stop growing or grow at a slower rate when they attain the sizes corresponding to the modal values. Within a given time, therefore, these nuclei appear with greater frequency. This finding is confirmed by comparing rhythmic growth of the nucleus, as indicated by caryometric statistical research, with the rhythmic growth of the nucleus in cells in cultures, measured at regular intervals with motion pictures (Wermel and Portugalow, 1935). A somewhat similar comparison between a statistical study of a dynamic phenomenon and the direct study of the same in living cells has been made by Möllendorff and co-workers (1937) in order to determine the relative length of the mitotic phases (see also W. Thompson-D'Arcy, 1942).

Some technical precautions must be taken, the first being that of geometric determination of nuclear size. In all tissues with spherical nuclei the problem is of course easy, but when we deal with ellipsoid-shaped nuclei the problem of the orientation of the axes is of the greatest importance and needs careful previous control. For the first type of cells the liver, testicle, and corpus luteum offer very good material; for the second groups the root tip cells, the cubical or cylindrical epithelia, and the smooth muscular cells of the uterine wall are very suitable.

All studies summarized here were conducted by drawing the nuclear outline with a camera lucida and measuring its diameters. The frequency curves were drawn and the modal values calculated. Since the curves are influenced by the growth of the nuclei they are not of the true "normal" type; mean, standard deviation, and median have no biological significance. Only the modal value is of biological interest because it reveals the steps during the growth cycle. The modal value of the volume can be calculated directly from the modal value of the diameter, which is not the case for other statistical parameters.

2. (*b*) *Physiological methods*

In the statistical study of nuclear variability, frequency curves sometimes have more than one modal value. Sometimes these modes are represented by very different frequencies, one mode appearing as principal and the others as secondary. If observed on isolated histograms the secondary modes may sometimes be considered statistically doubtful because they are determined from very scant data. However, if we consider the histograms of the same tissue under different physiological conditions, we can frequently observe that what is a secondary mode under one condition may became a fundamental one in another physiological status.

Considering the histograms of various physiological conditions as a whole, we can not only confirm the statistical consistency of the small secondary modes of each physiological status, but also interpret the cyto-physiological significance of the nuclei that constitute each mode. This system of studying the caryometric variability of a tissue physiologically enables us to give biological value to certain data, which the purely statistical study of a single tissue could not do.

The modes that correspond to the volumes of the prophase are particularly important. These volumes belong to nuclei whose genes—and therefore chromosomes or chromonemata also—have completed a duplicating cycle and effectively represent a basic stage of interphasic growth, even though the prophasic nucleus shows some conditions of variability that affect caryometric measurements more than the interphasic nucleus (ellipsoidic form not oriented, larger imbibition phenomena, etc.).

The fundamental observation of Jacobj that the values of the maximum of frequency of nuclear sizes are in the relation 1:2:4:8, etc., leaves no doubt that the material constituents of the nucleus reduplicate at each cycle of growth, and therefore the phenomenon is related to the process of reduplication of the genes which constitutes the basic occurrence of the interphasic period. The nuclei belonging to the higher multiples of the volume are polyploid or with polytene chromosomes (Biesele, Poyner and Painter, 1942).

In our studies we are attempting to extend knowledge of the phenomena in the following manner:

First we tried to find out whether the nuclei that have no interphasic growth and have differing numbers of chromosomes (such as the meiotic elements) have a corresponding volume for each number of chromosomes.

Secondly we tried to ascertain whether the interval of rhythmic growth during which the nucleus duplicates its volume corresponds to an interphasic growth period, which begins with the post-telophase of the preceding division and ends with the prophase of the subsequent one.

Thirdly we tried to use special physiological conditions which would arrest nuclear reproductive activity and then cause it to begin again simultaneously in all nuclei under new, experimentally controlled conditions.

The statistical variability of these nuclei suggests that the growth obeying these physiologically stimulating or arresting conditions is of the "rhythmic" type.

With these three elements—comparison between nuclei without interphasic growth and with different numbers of chromosomes, comparison between the stages of rhythmic growth and the prophases, and the induction of simultaneous interphasic growth in all the nuclei of a tissue by means of controlled physiological stimulation—we can give a true significance of rhythmic growth in terms related to the duplicating processes of the nuclear genes.

2. (*c*) *The caryometric analysis of spermatogenesis*

The first question to be settled is whether in cells which have no interphasic growth and have a known but different numbers of chromosomes, the nuclear volumes have a constant value in proportion to the number of chromosomes.

The spermatogenetic series gives favorable results here, since the spermatocyte of the first order, the spermatocyte of the scond order, and the spermatid generally have chromosomes (or genomes) that are in the ratio 4:2:1.

The corresponding volumes of their nuclei, as has been known since the first studies of Jacobj (Jacobj, 1926; Freerksen, 1933; Hertwig, 1933; Sauser, 1936; etc.), are strictly in the same relation 4:2:1.

In certain cases the relationship is not the same (Wermel: Lepidoptera; Hertwig, Schreiber: Vertebrata), and we must consider these cases separately because they probably have different stages of endomitotic growth or some phenomena of chromatin elimination.

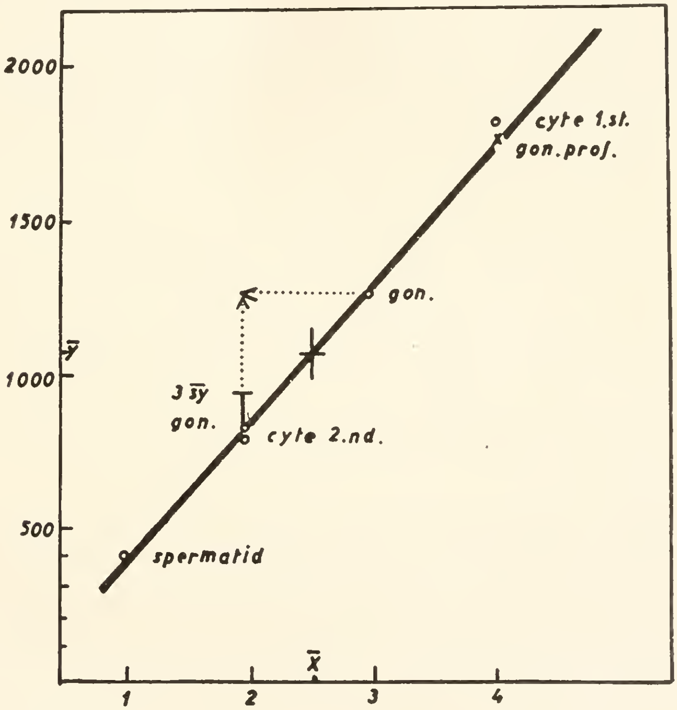

FIGURE 1. Scatter diagram and regression line between modal values of nuclear volume of spermatogenetic stages and the theoretical multiple values of the genome. From the average of 15 species of snakes.

It is important to stress here that the meiocytes ready for division represent a series of nuclei in which the multiple value of the genome is entirely proportional to the multiple value of the nuclear volume.

In the course of studies on meiocytes of snakes (Schreiber, 1946–48) we have verified this fact in 15 different species of neotropical Ophidia. The correlation between nuclear value and multiple value of the genome is always perfect, and the differences between the theoretical values and the actual ones are statistically insignificant.

We can therefore accept in testicular tissue the meiocyte series as a standard series of nuclear sizes that allows us to determine the quantitative value of the genome on the basis of nuclear volumes. This fact must be recognized as being limited to this tissue and no generalization made, for there are conditions in other tissues and organisms under which the correlation between genome and nuclear size does not follow the same rule (Wettstein, Dobzhansky, Barigozzi: see Schreiber 1946 c).

In the testicle, however, we find another category of cells, the spermatogonia, closely related to the meiocytes, but presenting a mitotic reproductive cycle with normal interphasic growth before the beginning of the meiotic process.

It is not our present task to analyze the growth phase which transforms the spermatogonium into a spermatocyte of the first order; these facts have been studied caryometrically in a short paper (Schreiber, 1948).

We will here analyze the mitotic cycle of the spermatogonium from the caryometric point of view by comparing the sizes attained by the nuclei of the spermatogonium during the interphase with the volumes just analyzed, of the meiocytes considered as standard size.

TABLE I

Nuclear volume in spermatogenesis (modal values)—average from 15 species of snakes

Cell	Spermatid	Spermatocyte 2nd order	Spermatogonium			Spermatocyte 1st order
			1st mode	2nd mode	Prophase	
Genome	n	2n	2n	(3n)	4n	4n
Volume	414	884	824	1271	1824	1765

The histogram of the nuclear volumes of a spermatogonium is generally unimodal, but in some cases presents a secondary modal value. These two modes are in a constant relative position, and from the study of many histograms (of 15 different species) we can conclude that the lower modal value corresponds to the volume of the second order spermatocyte, and hence to a resting diploid nucleus. The higher mode, which is generally the main mode, having a higher frequency value than the other corresponds to a volume that is 1.5 times the volume of the first mode, i.e. half way between the mode of the spermatocyte of the second order and the spermatocyte of the first order.

In the regression line between the modal values and the number of genomes (Fig. 1), the main mode of the spermatogonium corresponds to a genome value of 3. The difference between the volume of the main mode of the spermatogonium and the mode of the resting diploid nucleus (secondary spermatocyte) is statistically significant (more than 3 Sy.).

The volume of the spermatogonial prophase, which represents the final stage of interphasic growth in the mitotic cycle, is very nearly the same as that of the first order of the spermatocyte.

With these indications we can try to consider the entire interphasic growth of the spermatogonium (from the caryometric point of view) as follows: the diploid nucleus grows during the interphase and reduplicates its volume when it reaches the prophase. During growth, however, it stops when it reaches a volume that is about one and a half times the initial one.

This relation (1:1.5) between the two modal values of the spermatogonial growth cycle corresponds to the "Zwischenklassen" which some authors (Brummelkamp,, Hertwig) describe in comparing the nuclear volumes of different cells, tissues, or species. In the case of the spermatogonium we can be sure that these intermediate classes in the statistical analysis belong to the growth phases of a single category of cells, as shown in the growth curves studied by Wermel and Portugalow in the motion pictures of cells cultivated in vitro. In our preceding papers we called this intermediate stage of interphasic growth—"sesquiphase."

The rise of common hystological methods has not enabled us to detect any morphological features in the nuclei that belong to this "sesquiphasic" size and would allow us to interpret the real significance of this intermediate step. At present we can only infer the existence of this phase by means of statistical analysis of the volumes of interphasic growth. More suitable material would perhaps reveal some morphological detail that would be useful. In the discussion of results, we shall return to the problem of the nature of the sesquiphasic step, which has been discussed in our previous papers (Schreiber, 1943, 1946, 1947).

2. (*d*) *The caryometric analysis of uterine tissues during the sexual cycle*

As explained before, we have tried to analyze interphasic growth of nuclei in tissues where a specific morphogenetic stimulation was obtained with a suitable dose of hormones. Cutting off the supply of these hormones (as in castration), and followed by intensive treatment with hormones of the gland that had been removed, enabled us to study nuclear growth and to arrest it at both ends of the growth cycle.

The uterus in mammals during the normal estral cycle and pregnancy provides a medium for studying the same process of synchronous cell proliferation under physiological conditions, and for comparison with the above mentioned experimental conditions (castration and estrogenic treatment of castrated animals).

In the course of our work, facts have appeared which facilitate the statistical study of interphasic growth and give us the full picture of its rhythm. Many problems in endocrinology arise and these are treated separately (Salvatore, 1948, a, b, c). We have limited ourselves here to analyzing the statistical and cytological side of the phenomena, correlating this with the results obtained in other fields in which interphasic growth has been studied (Schreiber, 1943, 1946 a, b, and c, and 1948).

The studies reported here were performed by measuring nuclear volume and analyzing its statistical variability using the same general methods as those mentioned above. The first layer of the uterine epithelium, the glandular cells, and the muscular fiber of the meiometrium were examined during the estral cycle and pregnancy in white rats, mice and humans. In the castrated animal, we studied nuclear volumes in the untreated female, and during experimental estrus induced by injection of estrogenics.

In all cases the nucleus was considered as a rotating ellipsoid, and only those parts of the tissue with the nuclei well oriented for measurement were studied. We give here some typical cases representing respectively an estral cycle, a castration, an experimentally induced estrus, and a pregnancy, which give us the clearest picture of rhythmic nuclear variation. A complete description of all cases, with histograms and numerical tables, is recorded in the papers by Salvatore and Schreiber (1947), Salvatore (1947–48), and Schreiber, Mello and Salvatore (1949).

The following facts were observed: During the period of diestrus all the nuclei are simultaneously at rest, at a basic volume which we conventionally call "1." During the period of increase in hormonic activity, the nuclei begin to grow, showing a rhythmic pattern (polymodal frequency curves) reaching double size after having stopped temporarily at the intermediate stage of 1.5 times initial size. The phenomenon is exactly the same in all categories of cells studied, and we believe that muscle cells are of special interest, since their cyclic growth has hitherto been completely unknown.

When the hormone reaches its maximum of concentration at estrus, the nuclei seem to stop simultaneously after reaching size 2, as though they were waiting for some new conditions which would allow them to begin mitosis. Some nuclei undergo further volumetric rhythmic growth and reach sizes 3 and 4.

During estrus and the succeeding short transitional stage (estrus-metaestrus) mitosis appears in many cells by a change in the hormonic conditions (the nature of which is still under discussion by physiologists, i.e. quantitative or qualitative), and statistical analysis of the nuclear sizes reveals the reappearance of lower volume categories. Some cells degenerate and are probably phagocytized; others begin a new growth cycle. At the end of this period, no mitosis is present (metestrus) and all the nuclei are once more resting at size 1, just as they were during the diestrus stage at which the cycle was begun (Fig. 2).

The morphological features of the nuclei during the phases of the cycle are slightly different. The nuclei of the initial stages are more likely stained and of more compact aspect than those of double size, which have one or two clearly visible nucleoli. These facts appear in both epithelial and muscular cells. An interesting modification in the morphological aspect of the nuclei has been reported by Pfeiffer and Hooker (1944) in the stromal tissue of the uterus of mice under different hormonic conditions, some of which can be compared with ours. It does not seem impossible that the features described by these authors belong to the phases of a typical "endomitotic cycle" (endoprophase, endometaphase, etc.), but the authors do not attempt any interpretation and do not give any detailed statistical record of the volumes.

During pregnancy there is a situation identical to that of the estrus stage, complicated in the rat by an intermediate cycle of cell division on the 13th and 14th days. Of special interest was the muscular layer, in which we found a number of cells continuing rhythmic growth and reaching high multiples of the basic volume. This condition is identical in the uterine segment bearing the foetus which is mechanically enlarged, as well as in the intermediate segment without foetus. This eliminated the idea of hypertrophy, of a mechanical origin, of the muscle cells, which some authors believe.

These studies elucidate from a quantitative point of view, the nature of uterine hypertrophy and hyperplasy. Rhythmic growth of the nucleus appears to provide a new explanation for the hypertrophy of cells during pregnancy and estrus growth, i.e., as being due to interphasic growth and subsequent division (hyperplasy), or interphasic growth without subsequent division but followed by many successive endomitotic cycles (true hypertrophy) (Salvatore, 1948 c).

In the untreated castrated animal the caryometric picture is exactly the same as that during diestrus and metaestrus; i.e., an absolute rest of all the nuclei at the basic volume 1. The experimentally induced estrus and the successive estrus-

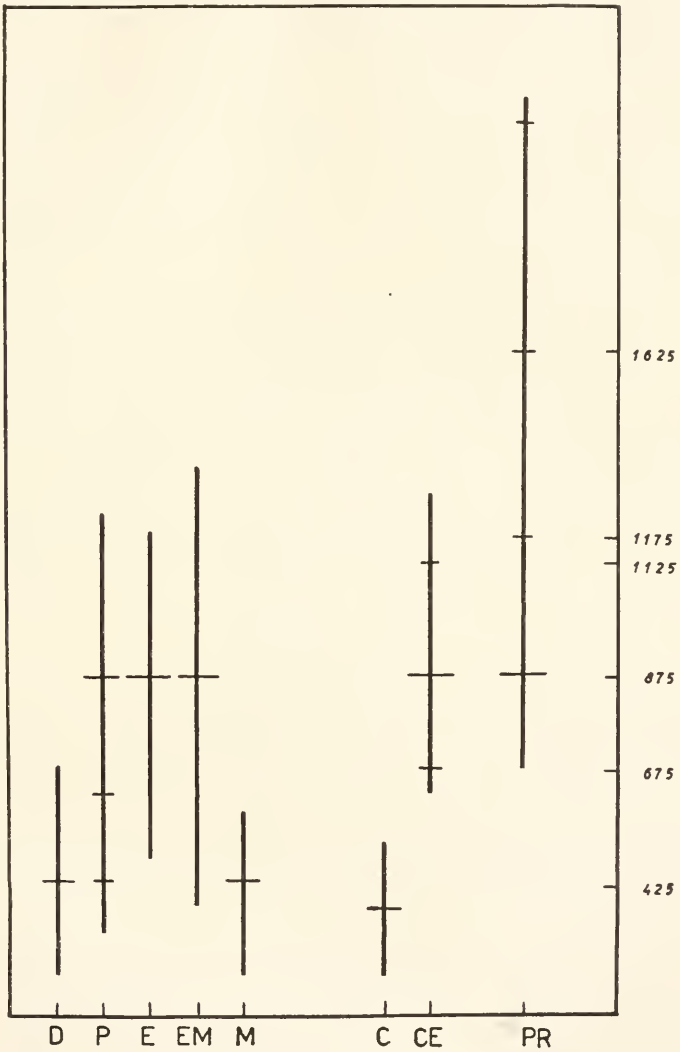

FIGURE 2. Nuclear sizes of rat miometrium during the stages of the estral cycle, castration, experimentally induced estrus, and pregnancy.

The vertical lines represent the total range of variation of nuclear volumes. The longer transverse lines represent the main modal values;' shorter transverse lines represent the secondary modes. Values on the right side of the diagram give the average of the modal values of all stages. D = diestrus; P = proestrus; E = estrus; EM = estrus-metestrus; M = metestrus; C = castrate; CE = experimental estrus in castrated animal; PR = pregnancy.

metaestrus stage obtained by the interruption of the hormone supply are identical, however, and clearer than the corresponding physiological stages.

We can infer from the above facts that the increase in hormonic concentration results in the volumetric increase of the uterine cell nuclei, and this fact manifests itself by a doubling of the volume one or more times. During physiological estrus and during the interruption of the hormonic treatment, mitotic activity appears, and the factors which induce mitosis find practically all the nuclei ready to begin the prophase and divide ("mitosebereit" of Hertwig).

The volume of the prophase (size 2) and that of the resting nuclei in castrated animals (size 1) give us the extremes of a duplicating cycle, just as the volume of the meiocyte gave the limits of interphasic growth of the spermatogonia.

This indicates the true interphasic nature of nuclear growth during the physiological phases of the uterus and the action of hormone stimulation, and can hardly be explained by a simple water imbibition or some colloidal modification of the cell, as some authors believe.

Comparing the total range of the histogram of the nuclear volume of the castrated rat and that of the rats experimentally maintained in estrus (Fig. 2), it appears evident, from the lack of any overlapping of the histograms, that there are not two or more categories of cells of different specific initial sizes, originally present in the tissue, as some authors believe; the statistically recorded modifications consist of a real interphasic growth of all or nearly all the cells simultaneously in the tissue.

In the uterine cell it is therefore possible to halt interphasic growth by means of specific endocrine conditions at both ends of the cycle. The intermediate steps during reduplication (sesquiphase) are revealed by the frequency curves with unusual clarity, and from the total absence of intermediate size classes in the castrated animals, we can infer that this sesquiphase is also a real growth phase and not a different category of cell size.

2. (*e*) *The caryometric analysis of ovarian endocrine tissues*

During the development of the graffian follicle, the cells of the granulosa layer undergo repeated mitotic divisions. We thus have here another homogeneous tissue in active multiplication, and we can apply the same general principle of the statistical analysis of interphasic growth.

After the bursting of the follicle, the transformation of the follicular cells is accomplished by enlargement of the nucleus and the cytoplasm. The statistical analysis of this phase gives us another clear picture of the rhythmic growth of the nucleus.

Figure 3 represents the modal values and the total range of variation of nuclear size in a developing follicle and in the corpus luteum. At the beginning of the follicle development, the oocyte is surrounded by only one layer of cells. The nuclei of follicular cells at that stage are predominantly at basic volume and some nuclei are at 1.5 times greater. In a more developed follicle the histogram shows three distinct modes: volumes 1, 1.5, 2 respectively. The prophases are all at size 2. We have here the same condition as previously stated in the uterus layers and in the spermatogonia. The growth cycle of the cell consists of a duplication of the nuclear volume showing the intermediate step (sesquiphase) and ending with the prophase.

The granulosa cells have a mitotic index of about 10 per cent during the period of the growth of the follicle, which drops to 0 per cent at the moment of luteal transformation.

The luteal cell of a transitory corpus luteum has a very regular statistical distribution of nuclear volumes with only one mode.

This mode corresponds exactly to the volume of the prophase (size 2) of the granulosa cells of the follicle. In the corpus luteum during pregnancy there is a further volumetric growth of some cells whose nuclei reach size 3, and probably 4 and 6.

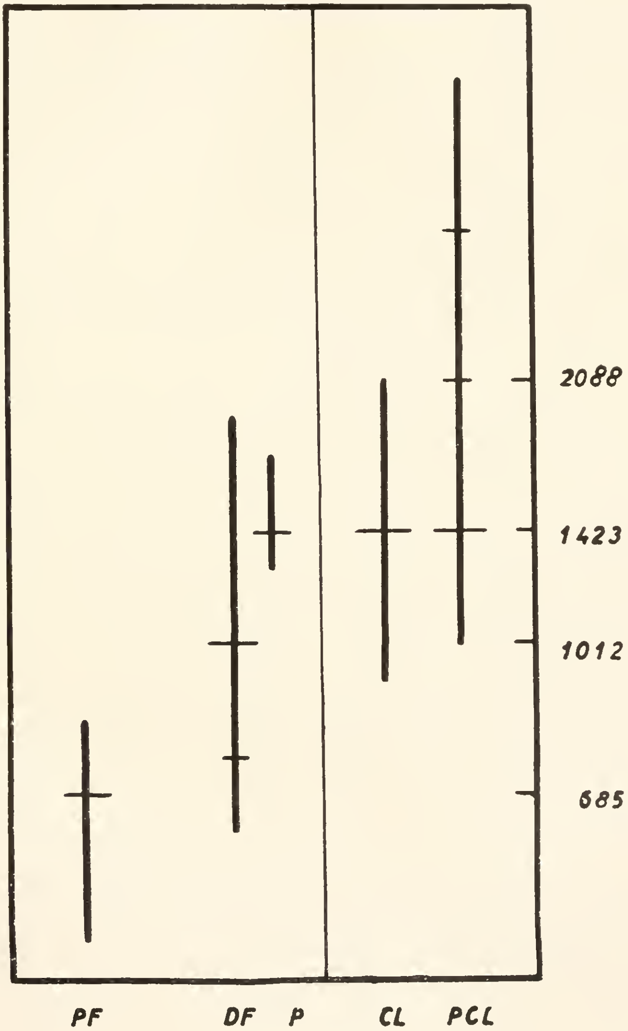

FIGURE 3. Nuclear sizes of the cells of graffian follicle and corpus luteus in the rat. Same explanation as Figure 2. PF = primary follicle; DF = developing follicle; P = prophases of developing follicle cells; CL = corpus luteus (cyclic); PCL = corpus luteus in pregnancy.

These higher multiple values of the phasic one are more difficult to establish owing to their rarity and the greater variability of the larger nuclei. There are no apparent differences in the morphology of these different classes of nuclei.

It is generally assumed that the luteal cells originate from the granulosa cells, although some authors believe that the cells of the "theca interna" of the follicle also contribute to their formation. The nuclear sizes of the cells of the granulosa layer and those of the luteal cells form a series of rhythmic values (Table II). This indicates the probable origin of the luteal cells from the granulosa; these reach the end of interphasic growth, and instead of beginning the prophase and dividing, continue the endomitotic growth and become transformed into the luteal cells under the influence of the proper hypophyseal hormone. These luteal cells can grow still further, with endomitotic cycles reaching rhythmic values higher than those of the initial size.

We must here recall the discussion on use of the term "endomitosis" in a broad sense, meaning reduplication of the nuclear genes without nuclear division. This reduplication may be accompanied by a reduplication of the number of chromosomes (true polyploids) or by that of the chromonemata within the chromosomes without variation of the original ploidy (polytenic chromosomes). Furthermore this reduplication of the nuclear content may be accomplished in some cases by the appearance of the morphological stages of Geitler's "endoprophase," "endometaphase," "endoanaphase" and "endotelophase," or in other cases without any morphological manifestation of the chromosome nucleinization cycle [as in the case of the ileum cells of the mosquito (Berger and co-workers)].

The luteal cells belong to the last category of cells, showing no visible variation of the inner feature of the nucleus in the different classes of size.

We should perhaps relate these results to Painter's studies on nuclear participation in the secretory cycle of cells (Painter, 1945). The increased need for cytoplasm ribosenucleic acid in the actively functioning cell is, as Painter thought, supplied by a reduplication of the nuclear genome and is manifested in the different types of glands by successive mitotic cycles or by endomitotic growth of the nucleus. We could perhaps raise the question as to whether this nuclear activity during the secretive processes might also explain the interphasic nature of the observed phenomena in such cells as the follicular and luteal, in which the chemical constitution of the secretion is not, at least, in the ultimate stage of proteic nature.

Summarizing the results of the statistical study of ovarian cells we can furthermore emphasize the fact that the rhythmic growth of the nucleus represents true interphasic growth, having an intermediate step at 1.5 times the initial size of each duplicating cycle.

3. Discussion and Conclusion

Before trying to describe the quantitative characteristics of nuclear growth during interphase, we must bear in mind that during this period, caryometric analysis can give only a rough, quantitative aspect of what occurs in the nucleus, and only by comparing the steps reached under different conditions and using material in which the internal characteristics can be studied can we try to draw some definitive conclusions.

It should not be forgotten that nuclear size is the result of a number of physical, physico-chemical, and chemical phenomena acting during the period in which the genes reduplicate and probably during which they perform their specific action in the cells. It is not definitely ascertained at what moment of the nuclear cycle reduplication takes place, although the interphase seems to be the most probable. We cannot affirm either whether gene reduplication and chromosome splitting are simultaneous, because they occur at different levels of molecular and morphological organization. Furthermore, we must limit our analysis to the simplest case of the interphase of somatic cells, and not extend it to the most complicated cases of auxocytic growth, post-meiotic divisions or segmentation of the blastomeres, in which other conditions (i.e., multiple strand constitution of the chromosomes) would complicate the analysis.

Caryometric studies with statistical analysis of the prepared tissue conducted at the same time as the motion pictures of the living nucleus (Wermel and Portugalow, 1935) confirm that rhythmic growth, as deduced from the modal values of the frequency curves of nuclear volume, corresponds to a real discontinuous growth. The modal values correspond to the steps reached after each growth period is ended. The studies conducted in the regenerating liver by use of colchicine methods (D'Ancona, 1939–41–42) and in the neoplastic tissues by statistical methods (Biesele, Poyner and Painter, 1941) confirm the close correlation between multiple modal values and polyploid or politenic status of the chromosome complement.

The experiments we have carried out in the spermatogenetic stages and in the uterine and ovarian cells might give a more complete picture of rhythmic growth, because of material and physiological conditions that permit the recognition of the true interphasic nature of that discontinuous growth.

We can summarize the facts as follows:

(1) Comparison of the volumes attained at the successive steps of the spermatogonium mitotic cycle, with the volumes at the stages of the meiotic elements (in the vertebrate testicle), allow us to measure variations in volume in terms of quantitative values of the genome.

(2) During the mitotic cycle the prophase represents the end of a growth cycle of the nucleus and corresponds physiologically to the completion of a reduplication cycle of the nuclear genomes. In all cases the volume attained during prophase corresponds to volume 2 of the rhythmic growth series, thus indicating the interphasic nature of the rhythmic steps.

(3) Using the physiological conditions of the sexual cycle in mammals we can study interphasic growth in the nuclei of a specific tissue sensitive to the stimulus of corresponding specific endocrine conditions. These conditions permit growth to be stopped at both extremes of the nuclear cycle. Here too the prophasic volume gives us the limits of a reduplication cycle of the chromosomes, and allows us to consider rhythmic growth as truly interphasic.

(4) The whole series of rhythmic values of nuclear size in all the tissues studied indicates that this growth is accomplished by a succession of reduplicating cycles, but is complicated by the existence in each reduplicating cycle of an intermediate phase ("Zwischenklasse") in which the nuclear volume is one and a half times the initial volume of each cycle ("Sesquiphase").

The ratios between the steps thus appear to be alternately 1:1.5 and 1:1.33, and the whole series of values should be 1:(1.5):2:(3):4:(6):8:(12):16 etc., the steps in parenthesis being the so-called sesquiphases.

These facts clearly appear from the scatter diagram of Figure 4. Table II shows the regression of the modal volumes in the theoretical rhythmic growth series with

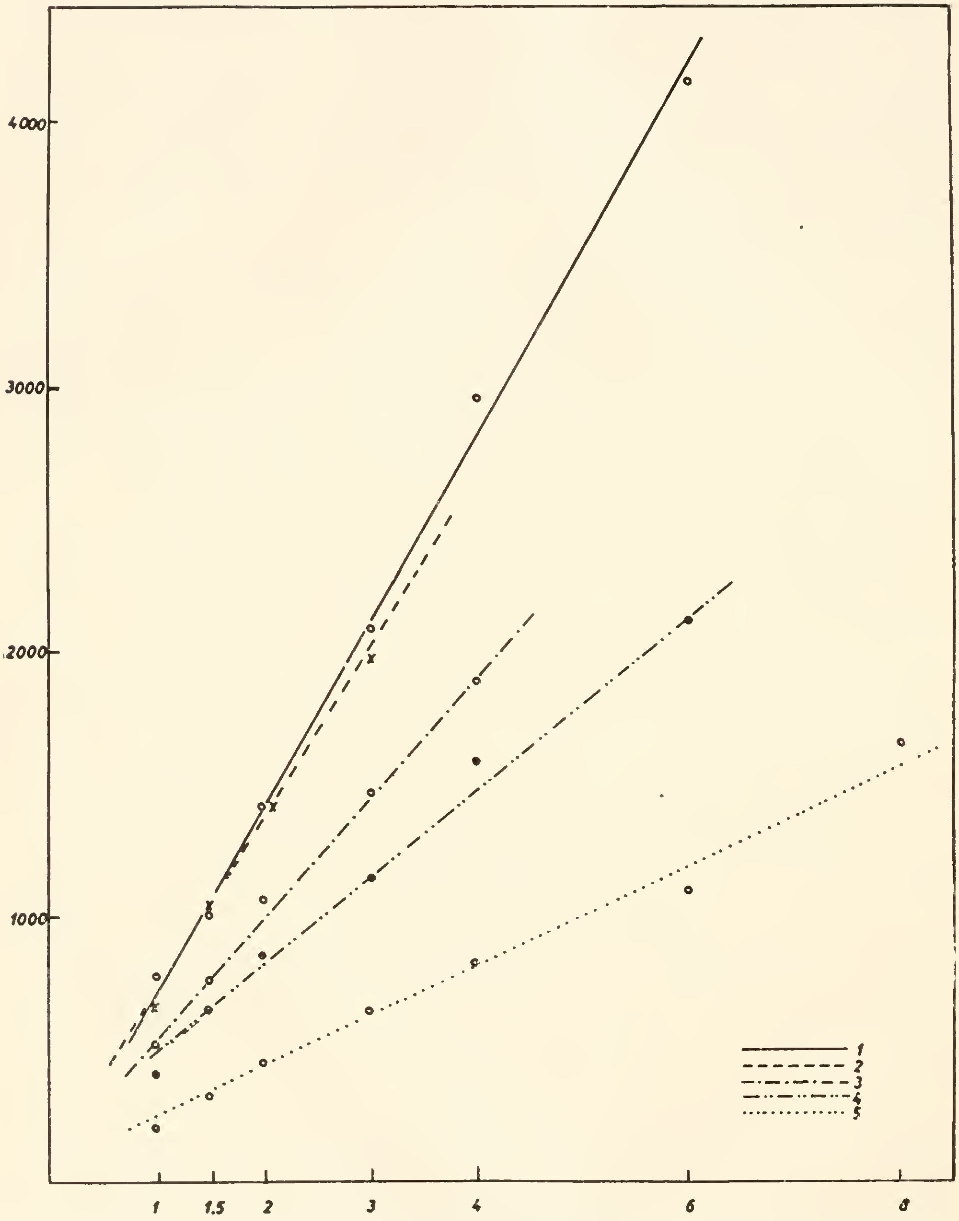

FIGURE 4. Scatter diagram and regression lines between modal values of nuclear volumes of uterine and ovarian cells and the theoretical series of the rhythmic growth stages with the intermediate step (sesquiphase).

(1) Graafian follicle and luteal cells in the rat. (2) Endometrium of the rat. (3) Human miometrium. (4) Miometrium of the rat. (5) Miometrium of the mouse.

the intermediate steps of the sesquiphase. We cannot discuss here the problem of th absolute values of nuclear volume in various tissues of an organism, which was considered in the original research of Jacobj, but which we believe should be further analyzed.

Rhythmic growth of the cell nucleus thus appears to be related to interphase activity. The size of the nucleus increases with a period of active growth, alternated with periods of rest. After reduplication of genic material is accomplished, generally the nucleus begins the prophase stage and divides. Sometimes mitosis is suppressed and a new reduplicating cycle (endomitosis) begins.

From the relationships between modal volumes and the multiple value of the chromosomes or chromonemata we must infer that at each step after a growth cycle, the nucleus consists of the genes and the material accompanying them which form the chromosomes, the nucleolus, and nuclear sap, the quantity and physico-chemical status of which determine, in regularly shaped nuclei, a nuclear size proportional to the number of genomes present in the nucleus.

Table II

Nuclear volume (modal values) of the uterine and ovarian tissues (P = prophase)

	Series of modal values of the rhythmic growth of the nucleus						
Theoretical series	1	1.5	2	3	4	6	8
Rat endometrium	665	1037	1460 1428 P.	2100 1983 P.			
Rat follicle and lutheal cell	685	1013	1423 1436 P.	2088	2961	4157	
Rat miometrium	420	651	863	1152	1592	2108	
Mice miometrium	216	337	444	642	830	1100	1650
Human miometrium	522	767	1078	1473	1891		

This principle makes it possible for us to understand the whole mass of facts revealed by caryometric analysis. We must consider as a fundamental fact the constancy of the ratio between the genes and the accessory materials that accompany them in the morphological constitution of the nucleus, both in quantity and in physico-chemical status, during the true "resting" condition between two successive reduplication cycles.

During the "metabolic" period (interphasic growth period) this ratio is obviously altered by the phenomena of water and material changes between the nucleus and the cytoplasm; but the ratio goes back to its initial value at the end of each cycle.

The nature of the intermediate step during the reduplication cycle, which we call "sesquiphase," is more difficult to understand. We do not know of any cytological feature of the nucleus specific for that phase; its evistence is only inferred from statistical analysis. We note that these steps do not appear in all tissues or in all species. The classical series of Jacobj and of many others give us a clear picture of a purely reduplication series.

Notwithstanding, in many other cases recorded by previous authors and in the ones here studied, as well as in the nuclear diminution series studied by Schreiber and Romano-Schreiber (1941) the existence of these intermediate steps is evident. It is not possible to call upon factors involving the geometric form of the nucleus in determining those steps, because they appear in both perfectly spherical nuclei (spermatogonium and luteal cell) and elliptical ones (endometrium and miometrium).

If we compare the histograms of the same tissue under different physiological conditions, we see that the same modal value always appears at the same position, and often what is the main mode in one physiological condition can be a secondary modal value in another. This fact allows us to consider the "sesquiphase," which statistics indicate to be a true biological phenomenon.

The theoretical explanation of the sesquiphase may be attempted by different methods and we can try to analyze some of them here. We might first look for a physiological explanation as G. Hertwig and Brummelkamp did. We can imagine some reasons for a stoppage or slowing down of the growth when approximately 50 per cent of the initial volume is attained. We cannot analyze here the complicated and perhaps artificial theories of G. Hertwig, based upon a hypothetical factor acting in different categories of nuclei, and correlating the chromosome number in some cases with the nuclear volume and in other cases with the nuclear surface. The mathematical explanations of Brummelkamp appear even more fantastic. Both authors consider the ratio between the steps as being 1 : 1.41, i.e., the square root of 2, which is very close to the ratio 1 : 1.5 considered correct by other authors. Wermel and his co-workers also consider the ratio to be 1 : 1.5. According to these authors the series should be 1 : 1.5 : 2 : 2.25. . . . For that reason these authors believe that the "Verdoppelungsgesätz" of Haidenhain and Jacobj must be rejected, but they do not give any new explanation of the nature of the rhythmic step of the growing nucleus.

We might also try to explain the "sesquiphase" in some other way, for example, by relating a different velocity of the splitting of euchromatic and heterochromatic regions of the chromosomes. Or we can take into consideration the different effects upon nuclear and nucleolar size of the two different types of chromatin (Fernandes and Serra, 1944). All this, however, would not explain the constancy of the ratio 1 : 1.5 between the steps in many categories of cells in which the sesequiphase can be detected, and which have a great variety of ratios of metero- and euchromatin.

Here it is interesting to note several facts found by Biesele (1940) relating to the 50 per cent increase in volume of the metaphasic chromosomes under certain physiological conditions without an apparent increase in the number of chromonemata. We cannot at present imagine what relation this may eventually have to the phenomena analyzed by us here, but we presume that these phenomena may eventually be taken into consideration.

Many other explanations could be offered in more purely speculative fields, such as for instance, different mathematical laws relating nuclear volume to the chromosome content in the different periods of the interphase, but here too, the constancy of the 1 : 1.5 ratio limits the possibilities of the hypothesis.

From a more genetic point of view, Heidenhain, since the very early studies of Jacobj, admitted that the intermediate values which in some cases appear as an exception to the "Verdoppelungsgesätz" could be explained by admitting that the two halves of the nuclear content derived respectively from maternal and paternal origin, reduplicate independently (Jacobj, 1931).

In the case in which only one reduplicates, the duplication ratio is not maintained. Hertwig (1937) admitted a similar point of view but subsequently rejected it without any justification, preferring the above mentioned theory of the two different factors acting on the surface or on the volume of the nucleus.

On the basis of the facts analyzed in this paper we can perhaps try to support more clearly this genetic point of view. We can admit (Schreiber, 1943) that in a diploid nucleus each chromosome set of different gametic origin represents a physiological entity during the reduplication process, and one set may be more precocious than another.

The influence of gametic origin on the behavior of an entire set or on some special chromosomes also manifests itself in other phenomena of the cell cycle (White, Schrader, etc.) : for instance, the precocious condensation of one haploid set in the scale insect, or the elimination of the paternal chromosomes in Sclara. A difference in the initial rhythm of reduplication between maternal and paternal chromosomes of each original pair is also invoked by Holt (1917) to explain the existence in Culex of the "six series" or "nine series" of chromosome numbers, in intestinal cells.

We could thus represent in an hypothetical way the so-called "sesquiphase" as a transitory stage in which one haploid set (or its multiples) has reduplicated and the other has not yet done so. The "quantum" of the simultaneous reduplication of the genes would in that case be the haploid set, or "genome."

All this is merely speculation which might perhaps lead to new research in a purely cytological field. As stated above we have at present no cytological evidence of the sesquiphase stage, which can only be detected by the statistical analysis of the growing nucleus.

As a general conclusion we can state that caryometric methods, when strengthened by the physiological conditions which specifically influence interphasic growth and mitosis, can help the cytologist to make a closer study of the growing nucleus and to formulate some suggestions on its quantitative aspect and on the dynamics of gene reproduction.

4. Summary

The author analyzes present knowledge on the problem of "rhythmic growth" of the nucleus as it appears from the point of view of statistical caryometric research. This analysis is carried out especially with regard to the problem of the intermediate steps during reduplication of nuclear volume to about 1.5 times the initial size.

The nature of these steps is analyzed experimentally in three different fields: (1) Interphasic growth of spermatogonia, whose nuclear size is compared with a meiocyte with a known number of chromosomes. (2) Interphasic growth of the uterine cells under different physiological conditions. (3) Interphasic growth of the granulosa cells of the ovary, and the transformation into luteal cells.

The interphasic nature of rhythmic growth is considered also as a possible explanation for the intermediate step during reduplication of nuclear size that is called sesquiphase.

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HOPKINSIAXANTHIN, A XANTHOPHYLL OF THE SEA SLUG HOPKINSIA ROSACEA

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Carotenoid pigments of land animals are usually one or more of the common, yellow constituents of their vegetable food (Zechmeister, 1934). By contrast, carotenoid pigments of marine animals, especially invertebrates, are seldom identical with the yellow constituents of marine plants (Fox, 1947; Lederer, 1940).

Another example of a carotenoid pigment found thus far only in a marine animal is the unusual rose-pink coloring matter of the nudibranch mollusk *Hopkinsia rosacea.* Hopkinsia is found in such small numbers along the coast of California that only one or two organisms, weighing but a gram or two, have been available at any one time. As a result, studies of the extracted pigment have been restricted to determinations of optical properties such as the characteristic spectral absorption curves, relative adsorbability in Tswett adsorption columns, phasic behavior and color reactions. These properties provide a basis for some deductions regarding the molecular structure of the Hopkinsia pigment. They indicate that this substance, which has not been described before, is a xanthophyll-like carotenoid. In accordance with widely accepted carotenoid nomenclature, this molluscan pigment is called hopkinsiaxanthin.

EXPERIMENTAL

Hopkinsia rosacea was collected at low tide at Moss Beach, San Mateo County, and at the Monterey Peninsula, Monterey County, California. When removed from its habitat, this organism proved to be exceptionally delicate and fragile; hence, specimens were brought to the laboratory in moist seaweed and placed immediately in about 100 ml. of methanol or ethanol. These alcohols removed all the pigment and yielded orange-yellow solutions. Pigments in these alcoholic extracts were transferred to petroleum ether. To this end, about 50 ml. of petroleum ether (B. P. 50–70°) and a large volume of water or of salt solution were added to the alcoholic solutions. When dissolved in petroleum ether, the extracted pigments formed a yellow solution in contrast to the orange-yellow color of the alcohol solution.

Adsorption of the petroleum ether solution of the extracted pigments on a column of powdered sugar (2.4 by 10 cm.) yielded a red-orange zone near the top of the adsorbent. When the adsorbed pigments were washed with petroleum ether plus 0.25 per cent n-propanol, most of the coloring matter moved rapidly through the column as a red-orange zone which contained the hopkinsiaxanthin. The colorless percolate below this red-orange zone indicated that carotenes and esters of hydroxy carotenoids were absent, because most of these substances are not adsorbed on columns of sugar. Above the red-orange hopkinsiaxanthin in the Tswett column, there appeared about five pale, indistinct, red-orange and yellow zones which were not examined further.

Hopkinsiaxanthin, contained in the principal red-orange zone in the adsorption column, was recovered by removal of the adsorbent with a spatula followed by elution of the pigment with alcohol. Readsorption of the pigment on columns of powdered sugar, of Celite and of activated magnesia always yielded a single band. These results indicate that a single pigment had been isolated (Strain, 1942, 1948). This same pigment was obtained from organisms collected in 1943, 1946, and 1947.

The physical and chemical properties of hopkinsiaxanthin are similar to those of the carotenoid pigments, particularly the keto carotenoids. As with the ketonic carotenoids, the color of solutions of hopkinsiaxanthin varies with the solvent. At equal concentration of pigment, solutions of hopkinsiaxanthin in nonpolar hydrocarbons are a lighter yellow than solutions in polar solvents such as alcohols. This effect, which is readily reversible with change of solvent, is illustrated by the spectral absorption curves reproduced in Figure 1.

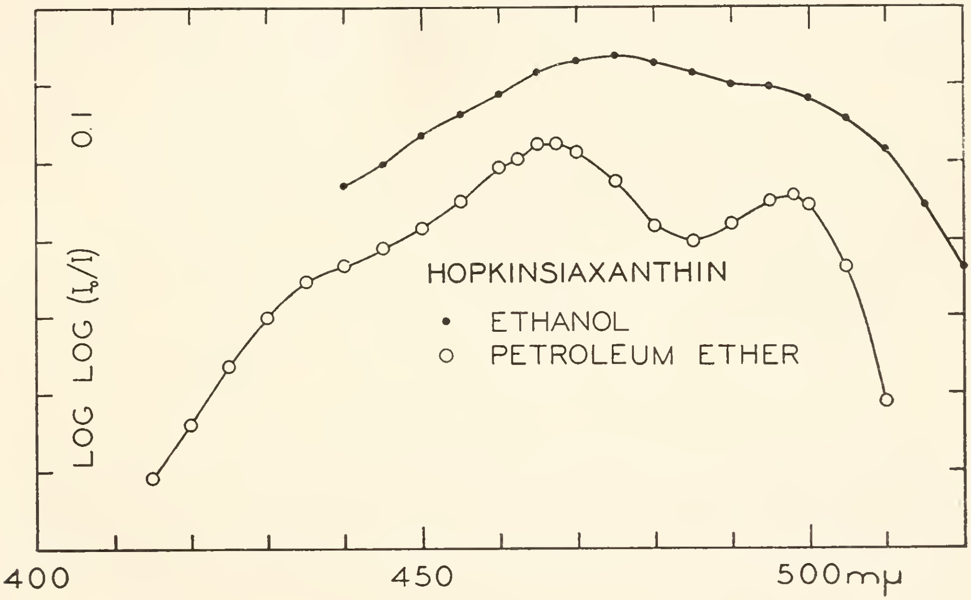

FIGURE 1. Characteristic spectral absorption curves of hopkinsiaxanthin measured in petroleum ether and in 95 per cent ethanol.

As shown by partition experiments, hopkinsiaxanthin is very much more soluble in 90 per cent methanol than in petroleum ether. With 80 per cent methanol, some of the xanthophyll dissolves in the petroleum ether, but most of it remains in the alcohol layer. With 70 per cent methanol, most of the pigment dissolves in the petroleum ether layer.

The adsorbability of hopkinsiaxanthin varies with the adsorbent and the solvent. This xanthophyll is strongly adsorbed on sugar or on Celite when petroleum ether is used as the solvent. It is but weakly adsorbed on these adsorbents when alcohol, petroleum ether plus alcohol or petroleum ether plus acetone are used as solvents. From these polar solvents the hopkinsiaxanthin is so strongly adsorbed on activated magnesia that acids must be added in order to elute the pigment. On powdered sugar and on Celite, the adsorbed pigment usually appears red-orange, but at low concentration it is salmon colored. Adsorbed on magnesia, the pigment is red-orange even when adsorbed at low concentration.

Adsorbability of hopkinsiaxanthin relative to some common plant carotenoids and chlorophylls also varies with the solvent as is illustrated in Table I. In columns of activated magnesia (plus siliceous filter aid), hopkinsiaxanthin is more tenaciously adsorbed than all the common xanthophylls including the very strongly adsorbed rhodoxanthin. Indeed, it is so strongly adsorbed that it cannot be washed along in the adsorption columns with the strongly polar solvents ethanol and acetone.. In this respect, hopkinsiaxanthin resembles the diketonic carotenoid rhodoxanthin, which is strongly adsorbed on columns of magnesia and but weakly adsorbed on columns of powdered sugar or of Celite (Strain, 1948).

TABLE I

Effect of different solvents upon the adsorbability of hopkinsiaxanthin relative to chlorophylls a and b, lutein and cryptoxanthin in columns of powdered sugar

Petroleum ether	Petroleum ether + 5 per cent acetone	Petroleum ether + 0.25 per cent *n*-propanol
Chlorophyll *b*	Chlorophyll *b*	Chlorophyll *b*
Chlorophyll *a*	Chlorophyll *a*	Hopkinsiaxanthin
Hopkinsiaxanthin	Hopkinsiaxanthin	+
Lutein	Lutein	Lutein
Cryptoxanthin	Cryptoxanthin	Chlorophyll *a*
		Cryptoxanthin

Hopkinsiaxanthin exhibits many color reactions that are characteristic of the carotenoid pigments. A solution of the Hopkinsia xanthophyll in diethyl ether shaken with concentrated hydrochloric acid yields a clear blue color in the acid layer, the blue color remaining unchanged for at least one-half hour. A petroleum ether solution treated with concentrated hydrochloric acid fades rapidly and forms a blue precipitate at the interface between the two liquids. A chloroform solution reacts with antimony trichloride yielding a blue color that also fades rapidly. Strong phosphoric acid (85 per cent) extracts all the hopkinsiaxanthin from petroleum ether and yields a blue color in the acid layer.

Strong alkalies convert hopkinsiaxanthin into a pale yellow pigment. For example, when a solution of this xanthophyll in petroleum ether is shaken with a 10 per cent solution of potassium hydroxide in water, the color changes from deep yellow to light yellow, although most of the pigment remains in the petroleum ether. When this petroleum ether solution is adsorbed on sugar, a weakly adsorbed, yellow band is formed. This yellow band is less adsorbed than the original, unaltered xanthophyll. Solutions of potassium hydroxide in methanol extract the hopkinsiaxanthin from the petroleum ether and the pigment fades rapidly.

Hopkinsiaxanthin dissolved in petroleum ether is but slightly affected by traces of iodine plus dimethylaniline (Strain, 1941). This reaction yields small amounts of pigments that are more strongly adsorbed than the unaltered xanthophyll on columns of powdered sugar.

DISCUSSION

The characteristic color reactions of hopkinsiaxanthin and the effect of polar and nonpolar solvents upon the spectral absorption properties (Fig. 1) suggest that this pigment may be a ketonic carotenoid. The weak adsorbability of hopkinsiaxanthin on columns of powdered sugar and the pronounced adsorbability on columns of magnesia (Table I) also support this conclusion.

As indicated by the wavelengths of the absorption bands, the hopkinsiaxanthin molecule contains about 11 double bonds with at least one of these in the form of a carbonyl group. The stability of the pigment toward solutions of iodine suggests that all these double bonds occur in the more stable, trans, spatial arrangement rather than in labile, *cis* configurations.

Because the adsorbability of hopkinsiaxanthin on columns of powdered sugar approximates that of lutein (dihydroxy alpha-carotene), it is possible that the molecule contains one or two hydroxyl groups. The preferential solubility of hopkinsiaxanthin in methanol relative to petroleum ether suggests that there are probably no esterified hydroxyl groups.

With respect to solubility and color reactions, hopkinsiaxanthin resembles only one of the principal carotenoid pigments isolated from marine plants; namely, fucoxanthin. However, the Hopkinsia pigment is not identical with fucoxanthin as shown by the wavelengths of the absorption bands: 466 and 497 mμ for hopkinsiaxanthin in petroleum ether (Fig. 1) and 449 and 477 mμ for fucoxanthin in petroleum ether (Strain, Manning and Hardin, 1944). It also differs from fucoxanthin with respect to adsorbability, for it is more strongly adsorbed than fucoxanthin on columns of magnesia, and it is less strongly adsorbed than fucoxanthin on columns of powdered sugar.

SUMMARY

The striking, rose-pink color of *Hopkinsia rosacea* is due to the presence of a carotenoid pigment, hopkinsiaxanthin. This xanthophyll, which has not been found in plants or in other animals, occurs in the stable, trans configuration and probably contains a carbonyl group.

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ANDROGENESIS, A DIFFERENTIATOR OF CYTOPLASMIC INJURY INDUCED BY X-RAYS IN HABROBRACON EGGS[1]

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INTRODUCTION

Any technic which differentiates cytoplasmic from chromosomal injury induced in the living cell by x-rays is of interest to investigators of biological effects of ionizing radiations. Injury to chromosomes is measured by their breakage and rearrangement and by visible and lethal mutations. Injury induced in the cytoplasm must, ordinarily, be studied by means of tests for physical and chemical changes in treated cells or by changes in behavior of the cell as a whole, inhibition of division, etc.

When evidence for the nature of effect is incomplete, it is always tempting to attribute greater sensitivity of egg than of sperm to cytoplasmic injury because of the extreme difference between these two types of cells in respect to amount of cytoplasm. Muller (1937) writes, "In Drosophila, a given irradiation of the eggs shortly before or after fertilization will result in non-development and death of a high proportion of them, whereas the same amount of treatment of spermatozoa alone allows more of the fertilized eggs to develop. Nevertheless genetic analysis of the resulting adults proves that they contain more genetic changes, *and more drastic ones,* in the latter case than in the former. The *difference* in death rate here, then, must have been of non-genetic origin, involving, no doubt, some injurious change in the egg protoplasm. On the other hand, the death of the embryos that were derived from treated sperm and untreated eggs must have been genetic." In experiments on irradiation of females of Drosophila, dose has not exceeded 6000 r.

Whiting, P. W. (1938) found that some eggs of Habrobracon survived a dose of 18,000 r (lethal dose for sperm in this species is about 10,000 r) and Whiting (1938) identified these as eggs irradiated in first meiotic prophase. Eggs treated in first meiotic metaphase were much more sensitive than sperm. This order in respect to size of lethal dose also holds for Sciara as reported by Metz and Boche (1939) and Reynolds (1941). In contrast to the results cited by Muller, then, eggs of Habrobracon and of Sciara are either more sensitive than sperm or less so according to condition of the chromosomes at time of treatment.

It is a generally accepted fact that the stage in the nuclear cycle is of importance in determining degree of sensitivity to x-rays and Muller in his epoch-making paper on artificial transmutation of the gene (1927) writes, "In addition, it was also possible to obtain evidence in these experiments for the first time, of the occurrence of dominant lethal genetic changes, both in the X and in the other chromosomes. Since the zygotes receiving these never develop to maturity such lethals could not be detected individually but their number was so great that through egg counts and effects on the sex ratio evidence could be obtained of them en masse." This refers to dominant lethal changes induced in the sperm.

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There is no argument, *a priori,* for the supposition that sperm have a monopoly on dominant lethal chromosome effects. The treatment of eggs in a nuclear stage which responds to relatively low doses of x-rays by the production of dominant lethal genetic changes will explain the greater sensitivity of eggs than of sperm.

The "genetic analysis of the resulting adults" to which Muller refers is an analysis of recessive or of non-lethal changes. Dominant lethal genetic or cytological effects are, of necessity, "strained out" in the process of reaching adulthood. As Sparrow point outs (in press), "In the presence of a low frequency of rejoining, an increased percentage of acentric fragments or deletions would be expected and thus a higher proportion of lethality would occur. Paradoxically, therefore, if one were scoring for aberrations in the F_1 generation, following radiation of one or both of the parental gonads, the recovered aberrations would not necessarily represent a true picture of total chromosome breakage since cells in the most sensitive stages would be the least likely to produce viable F_1's."

Habrobracon eggs vary greatly in their response to x-rays according to stage at time of treatment, and in the most sensitive stage studied (four times as sensitive as the sperm), cytological observation of large numbers of them after treatment has shown that death is due to chromosomal injury. The chromosomes of Habrobracon ($2n = 20$) are small, however, and any evidence for these conclusions gained from a different method of approach is of value and should help to convince those accustomed to work with large chromosomes who tend to be skeptical of observations made on small ones.

Androgenetic males, developed from the untreated sperm nucleus in x-rayed cytoplasm, furnish material for this different method of approach. A perfectly normal, fully fertile individual must, of necessity, have developed in an egg with cytoplasm not seriously altered by its exposure to x-rays.

Material and Methods

Wild type stock (No. 33) of the parasitic wasp, *Habrobracon juglandis,* with a hatchability of 96–98 per cent, has been used for all experiments since the beginning of this study in 1937.

Homozygous females of this stock were x-rayed and mated to untreated males with one or more traits recessive to wild type. These females were placed with host caterpillars, and their eggs were studied in respect to hatchability and/or were allowed to mature. Viable unfertilized eggs develop into wild type, gynogenetic males (both chromosomes and cytoplasm irradiated). Viable fertilized eggs develop into wild type, heterozygous, biparental, diploid females (chromosomes half irradiated, half not, cytoplasm irradiated). Fertilized eggs most seriously injured in respect to egg chromosomes develop into recessive, androgenetic, haploid males (chromosomes not x-rayed, cytoplasm x-rayed) (Whiting, 1946a). The present paper is concerned primarily with the two types of males. X-ray induced changes common to both afford proof for cytoplasmic injury.

Eggs were separated according to time of laying into those irradiated in first meiotic metaphase (metaphase I) and those treated in first meiotic prophase (prophase I) (Whiting, 1945a).

For x-ray treatments a dual-tube self-rectifying outfit with a simultaneous cross-firing technic was used. The secondary voltage was 182 kv. and the tube current on each tube was 25 ma. The heavy glass of the tube walls and 5 mm. of bakelite of the tube shields gave the filtering value of 0.2 mm. copper shield. The output intensity was 7210 r per minute, distance 9.5 cm. Females were placed in gelatine capsules for treatment. All breeding was carried on at about 30° C. Lethal dose [2] is the lowest dose used after which no eggs have hatched.

When dose was fractionated, it was divided into three periods of approximately equal length. Two-hour intervals were allowed between exposures for experiments on hatchability, one-hour intervals for those on androgenetic males.

OBSERVATIONS

In order to demonstrate the significance of new data, some results, previously published, must be summarized briefly. Studies (Whiting, 1945a) on hatchability of 6824 eggs x-rayed in metaphase I have demonstrated that, when they are unfertilized, (a) the survival cure is exponential, (b) lethal dose is about 2400 r, and (c) hatchability is not changed significantly by variation of intensity of treatment, fractionation of dose or delay in oviposition. When these eggs are fertilized after treatment by untreated sperm their hatchability is not significantly changed. It was concluded, therefore, that death is due to dominant lethal effects which arise from single irreversible events. Eggs, irradiated in this stage, and fertilized by untreated sperm, may give rise to diploid females if dose is sub-lethal or to an occasional androgenetic male at either sub-lethal or lethal doses (Whiting, 1946b).

Cytological study (Whiting, 1945a) shows that chromosome fragments (terminal deletions?) may be present in the first meiotic division after treatment in metaphase I, that these fragments increase in number with increased dose, and that chromatin bridges (resulting from sister-chromatid union?) are present in the second meiotic division. These bridges are permanent and, since the egg pronucleus remains attached to them, it is pulled into a "tear-drop" as it moves inward. In unfertilized eggs, it undergoes cleavage and, in fertilized eggs, it usually contacts the male pronucleus and fuses with it. The diploid nucleus then divides and shows clearly the difference between the two groups of chromosomes, treated and untreated. In both cases, chromatin bridges are present in cleavage divisions and death of embryos ultimately occurs. Occasionally, however, the egg pronucleus is retarded to such a degree that the sperm pronucleus divides without it and a normal, haploid androgenetic male is formed (Whiting, 1948). Androgenesis results, then, from structural changes in the x-rayed maternal chromosomes of the type which, when less extreme, cause death to gynogenetic males and to females.

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[2] Apparent inconsistencies in lethal doses in successive papers dealing with Habrobracon eggs are due to changes in method of calibration at the Marine Biological Laboratory. Conditions of treatment have not varied. In this paper all doses have been corrected for the latest measurements.

Hatchability studies on 12,634 eggs irradiated in prophase I (Whiting, 1945a) have demonstrated that, when they are not fertilized, (a) the dose-action curve is exponential only at lower doses and that, after higher doses, response increases at a disproportionate rate, (b) lethal dose is about 54,000 r, and (c) hatchability is not changed significantly by variation of intensity of treatment or delay in oviposition but is increased at high doses by fractionation of dose.

Prophase I eggs, free from dominant lethal changes after exposures to sub-lethal doses of x-rays, and fertilized by untreated sperm, develop into females. No androgenetic males have been produced by them. Cytological study of divisions after treatment in this stage has shown (Whiting, 1945b) that fragments, bridges, or both may appear in either meiotic division but that bridges, rarely present in the second meiotic division, are single and do not retard the egg pronucleus. No androgenetic males develop in these eggs, therefore, because of the absence of mechanical hindrance to free movement of the egg pronucleus. Even after very heavy treatments, eggs often fail to show any chromosome aberrations.

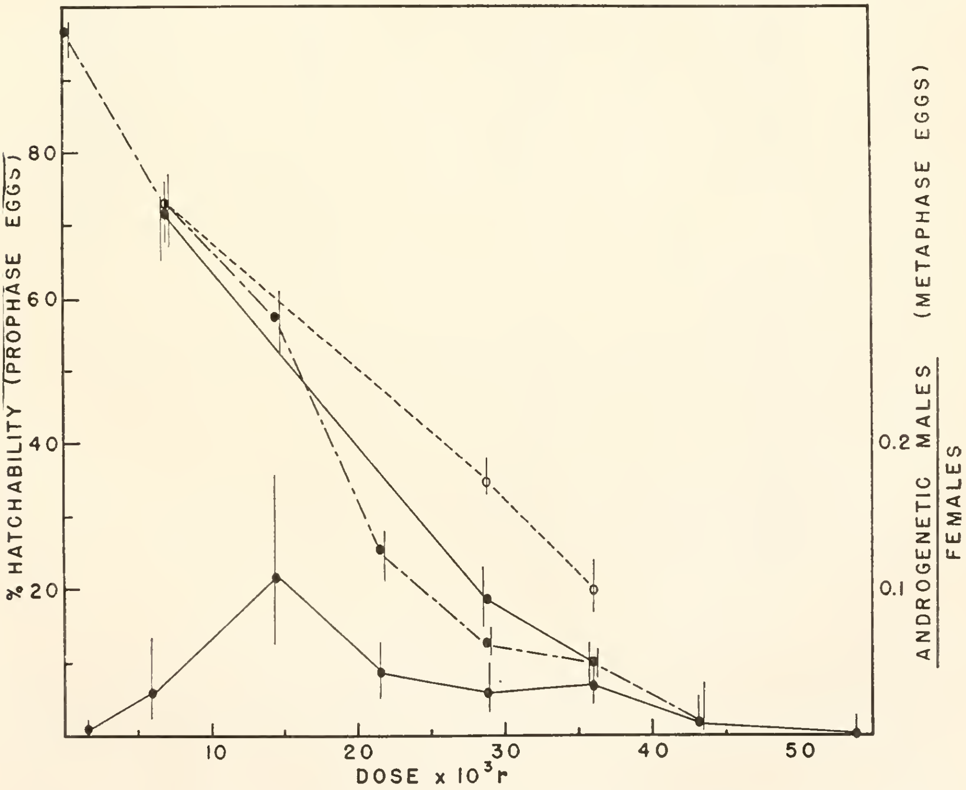

FIGURE 1. Lowest curve, $\frac{\text{androgenetic ♂♂}}{\text{♀♀}}$, plotted against dose. Remainder are dose-action curves for hatchability of unfertilized prophase I eggs. Continuous treatments (●), two experiments (—·—·—·) and (————); fractionated treatments (○), (– – – –). 95 per cent confidence interval is indicated for each experimental value.

Lowest dose after which significant reduction in hatchability was recorded was 50 r for metaphase I and 850 r for prophase I.

In Table I are assembled the data on the occurrence of androgenetic males in relation to dose. Results produced under constant experimental conditions are summarized in section A of the table and it is these which are expressed graphically in Figure 1. To be noted especially are the facts that (a) although androgenetic males develop only in eggs x-rayed in metaphase I (lethal dose for egg nucleus about 2400 r), they occur after treatment with doses up to that lethal for prophase I eggs (lethal dose about 54,000 r), and (b) that androgenetic males per treated female increase with dose up to about 15,000 r and then decrease, and (c) that fractionation of dose permits development of androgenetic males at doses lethal to them when dose is administered continuously. Data on fractionation and androgenesis are not extensive but in each of two experiments one androgenetic male was produced in comparison with none from 181 females treated with continuous dose. In two experiments listed in section B a similar difference between eggs exposed to continuous and fractionated treatments was found. Dose was not accurately measured but was probably about 50,000 r.

Table I

Androgenetic males in relation to dose and number of females x-rayed. A. Females from wild type stock No. 33, experimental conditions carefully controlled. B. Miscellaneous females, experimental conditions not controlled.

	Dose in r units	No. ♀♀ treated	No. androgenetic ♂♂	Androgenetic ♂♂ / ♀♀ Treated
A	675–2700	1015	5	0.0049
	6000	192	6	0.0313
	14,420	146	16	0.1095
	21,630	469	20	0.0426
	28,840	487	15	0.0308
	36,050	533	19	0.0356
	43,260	358	4	0.0111
	54,075	344	1	0.0029
		3544	86	0.0243
	64,169–144,200	359	0	
	64,169	181	0	
	64,890(frac.)	97	2	0.0206
		4000	88	
B	6000	65	1	
	12,000	150	4	
	14,420	623	6	
	40,000?	52	3	
	50,000?	89	0	
	50,000?(frac.)	87	8	
	Total	5066	110	

The chance that the array of ratios in section A, column 4, is a random variation of uniformity in expectation is infinitesimal according to the χ^2 test ($P = 0.000000$).

Facts relevant to the discussion of cytoplasmic injury are expressed graphically in Figure 1. They fall into two categories, those related to hatchability of unfertilized eggs x-rayed in prophase I (x-rayed chromosomes in x-rayed cytoplasm) and those related to incidence of androgenetic males (untreated chromosomes in x-rayed cytoplasm). The 95 per cent confidence interval for each experimental value is plotted in the figure. Tables of confidence of Ricker (1937) and of Clopper and Pearson (1934) were used.

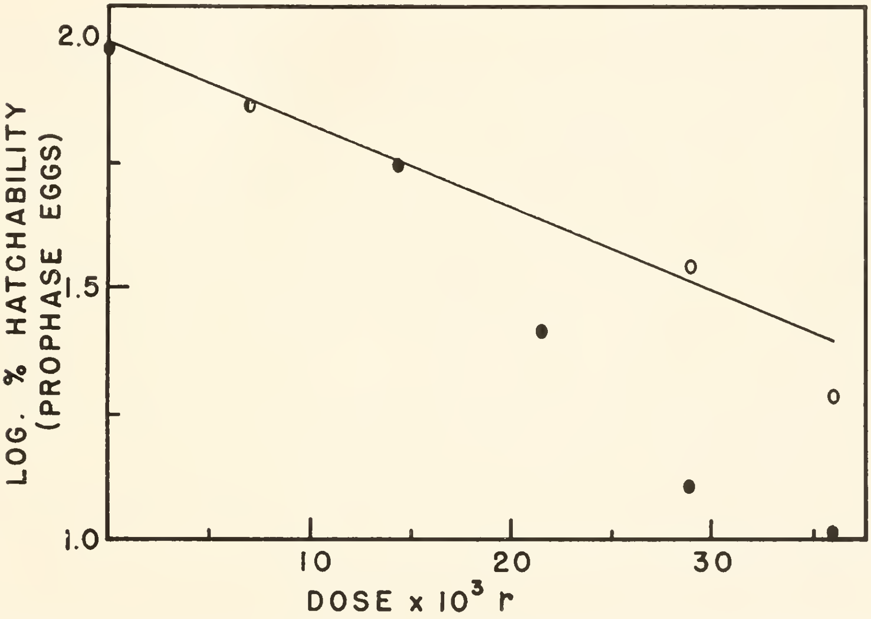

FIGURE 2. Percentages of hatchability of unfertilized prophase I eggs plotted semilogarithmically against dose; control and continuous treatments (●), fractionated treatments (○). By method of least squares a straight line was fitted to data of untreated, one minute (continuous + fractionated), two minutes (continuous), four minutes (fractionated) and five minutes (fractionated). For significance of experimental values consult Figure 1.

For hatchability of eggs x-rayed in prophase I with continuous treatment, there are two dose-action curves; with fractionated treatment, one. The former curves are not exponential, the latter is (Fig. 2). Note that the dose at which response to fractionation becomes apparent is about 15,000 r.

The curve representing androgenetic males per female rises to about 15,000 r and then falls until the lethal dose for them is reached. A steadily increasing number of chromatin bridges with resultant increase in retarded egg pronuclei is to be expected with increase of dose, so that androgenetic males should increase steadily with dose if there were not a concomitant increase of some factor which reduces their viability. Since this factor is x-ray induced, it must be cytoplasmic.

Several questions may have occurred to the reader and these are discussed at this point.

Why are there so few androgenetic males, even at doses optimal for their occurrence? Is there a high mortality of androgenetic embryos and, therefore, some permanent cytoplasmic injury at all doses? A comparison of androgenetic males per metaphase I eggs laid (6/381) with androgenetic cleavage per metaphase I eggs studied cytologically (6/291) shows that the difference between these two groups is not significant at the dose ranged used, 14,420 – 28,840 r. The small number of androgenetic males is due, then, not to the death of many but to the fact that very few egg pronuclei are sufficiently retarded to permit development of sperm nuclei alone (Whiting, 1948).

How do androgenetic males compare in vigor, viability, fertility and mutation rate, with gynogenetic males produced by the same females and with untreated controls? Detailed data on this subject are being prepared for publication, but in summary it can be stated that there is a striking difference between androgenetic males and gynogenetic males from the same females. All but one of the 110 androgenetic males found have been perfectly normal, vigorous, and fully fertile. The single exception died as a pupa. No visible mutations have appeared in them. They are indistinguishable from untreated controls in every way. Gynogenetic males tend to die as larvae or pupae and survivors have shown a significantly higher percentage of visible mutations, 29 among 417 or 6.95 per cent. Using the exact method of treating contingency tables (Yates, 1934), it is found that the chance that these percentages, 0 and 6.95, are variants of uniformity in expectation is very low ($P = 0.00188$).

Do heavily irradiated females lay fewer eggs than those treated with lighter doses? If so, this would reduce ratio of androgenetic males to mothers. Detailed analysis (Whiting, 1945a) showed that, after any dose up to 150,000 r, the average number of metaphase I eggs per female is the same as for controls.

Do sperm enter heavily irradiated eggs as freely as controls and do they move about in the egg normally? X-rayed females mate readily after any dose and, after sub-lethal doses, produce the expected ratio of daughters.

Are the cytological phenomena of the x-rayed egg nucleus the same at very high doses as at low? No accurate counts have been made of relative numbers of fragments, bridges, retarded pronuclei, etc., at very high dose but all these conditions have been observed in many eggs after treatment of metaphase I with 60,000 r.

Discussion

Evidence for chromosomal injury as the cause of death of Habrobracon eggs irradiated in metaphase I, with doses up to 2400 r, is convincing. The survival curve is exponential, and cytological study has demonstrated that ootids without chromatin fragments also give an exponential dose-action curve (Whiting, 1945a; Lea, 1946). This curve is higher than actual hatchability and suggests that some ootids without fragments are inviable. This can be explained by failure to see small fragments in some eggs. Such a failure is understandable in view of the small size of Habrobracon chromosomes and, therefore, still smaller size of the fragments and the granular nature of the yolk in which they lie. It is highly probable that each egg that fails to hatch has at least one fragment in it.

That death of x-rayed metaphase I eggs is due to chromatin loss through terminal deletions which act as dominant lethals (Muller, 1940; Pontecorvo, 1941) has been accepted by Lea (1946) and he discusses the similarity of these results to those of Sonnenblick (1940) on Drosophila eggs. Lea writes, after a detailed discussion of the subject (1947), "It is evident that dominant lethals in unfertilized eggs, as well as in the sperm, can be explained by lethal types of structural change." From the data on hatchability of x-rayed metaphase I eggs of Habrobracon, he estimated that there are 1.7 breaks primarily produced per 1000 r per haploid chromosome complement and that all breaks are permanent (Lea, 1946). This accounts for the extreme sensitivity of these cells by a method consistent with facts and with theories accepted for the explanation of similar responses of sperm cells to x-rays.

Evidence for causes of death of Habrobracon eggs irradiated in prophase I, with doses up to 54,000 r, suggests that both chromosomal and cytoplasmic changes are involved. The study of the nature of chromosomal injury is complicated by the type of dose-action curve which is exponential only for the first three points (including controls) (Fig. 2). There is no response to fractionation at these doses. Eggs, exposed to lethal doses, have been seen with chromosomal aberrations although many seem to lack them. This does not preclude their presence, however. The conditions of the chromosomes at time of treatment would allow for many types of chromosomal rearrangements, and their identification is more difficult than that of the relatively large fragments seen after irradiation in metaphase I. The increased response of x-ray prophase I eggs to higher doses suggested induction of a new phenomenon at about 15,000 r. This was interpreted (1945a) as due to an increase in multiple-hit, complex rearrangements which would increase rapidly at higher doses (Sax, 1938) and complicate the curve. Many types of multiple-hit chromosomal changes are viable, however, and would not be expected to reduce hatchability to the extent indicated in these experiments.

If the rise in mortality of both androgenetic and gynogenetic males induced by doses above 15,000 r were chromosomal in nature, it would be necessary to postulate that injury (breaks) in metaphase chromosomes decreases while that in prophase chromosomes increases above that point in response to increased dose. There remains the possibility that stickiness of chromosomes replaces breaks but this would increase androgenesis since it is permanent after high doses in the forms in which it has been reported (Sax, 1942). It is unlikely to occur in prophase chromosomes. There is no evidence for it in Habrobracon eggs irradiated with doses up to 60,000 r. If cytoplasmic injury is not involved it would also be necessary to suppose that fractionation of dose increases injury in metaphase chromosomes but decreases it in prophase chromosomes. One-hit chromosomal breaks (terminal deletions?), characteristic of metaphase injury, do not respond to fractionation of dose.

The first appearance of change in response to dose at about 15,000 r, its effect on reducing survival of gynogenetic and androgenetic males, its response to fractionation in increasing both types of males, combine to indicate that this change is due to cytoplasmic injury.

In Figure 2 are plotted semi-logarithmically percentages of hatchability of unfertilized x-rayed prophase I eggs. By the method of least squares a straight line was fitted to the data giving hatchability of untreated, one minute (continuous and fractionated), two minutes (continuous), four minutes (fractionated) and five minutes (fractionated) of treatment. For the last, percentage of hatchability is below expectation on the basis of one-hit permanent changes. One fraction of treatment at this dose was longer than any others used and perhaps this reduced recovery. Curve for hatchability after continuous treatments at high doses is clearly not exponential. All data suggest that, if proper conditions of fractionation are found, a good exponential curve of hatchability can be obtained which will represent the effects of dominant one-hit irreversible chromosomal changes only since cytoplasmic injury will be prevented. Under such conditions, the number of androgenetic males should increase steadily with increase in dose and percentage of visible mutations should be the same for both continuous and fractionated treatments. Lethal dose for prophase chromosomes should be over 90,000 r.

X-ray induced chromosomal changes have been explained by some investigators as "direct-hit" changes due to the production of ionization in particular molecules and this is known as the "target theory" (Giese, 1947; Lea, 1947). Criteria of the validity of this theory are (a) independence of response to change in intensity or to fractionation, (b) absence of a threshold for response, (c) dependence of response or wave length and (d) an exponential survival curve (for one-hit aberrations). Results of irradiation of metaphase I eggs clearly support this theory. Unfortunately, no experiments have been carried out with radiations of different wave lengths.

When cytoplasmic injury is eliminated by fractionation at high doses, the resultant phenomena likewise support the target theory of chromosomal change in eggs x-rayed in prophase I.

In contrast to chromosomal change, cytoplasmic injury has a rather high and definite threshold.

Does the cytoplasmic injury in itself prevent the androgenetic embryo from maturing or does it influence the untreated chromosomes so that they are incapacitated? Untreated male pronuclei in cytoplasm x-rayed with 70,000 r look normal. The consistent absence of any injury in surviving androgenetic males favors the view that cause of death of the embryo is directly cytoplasmic.

Two facts have been noted about heavily irradiated eggs: (1) when laid they are softer and more flexible than control eggs and (2) the first cleavage nucleus, whether haploid or diploid, tends to be situated more posteriorly than in control eggs. These facts suggest decrease in cytoplasmic viscosity.

To this author, the situation which exists at four minutes of treatment, 28,840 r, represents an ideal one for testing effects of environmental changes on cytoplasmic injury and thereby, perhaps, obtaining some clue as to its nature. Any response similar to that of fractionation of dose would indicate reduction of cytoplasmic injury.

SUMMARY

Wild type Habrobracon females were x-rayed and mated to untreated recessive males. Two kinds of haploid males were produced, gynogenetic (x-rayed chromosomes in x-rayed cytoplasm) and androgenetic (untreated chromosomes in x-rayed cytoplasm).

Eggs x-rayed in metaphase I × untreated sperm

Gynogenetic males develop from the unfertilized eggs, androgenetic males from fertilized. Death of the former and origin of the latter are caused by different degrees of the same type of x-ray induced chromosomal injury. Dose-hatchability curve for gynogenetic males is exponential and their lethal dose is that of the chromosomes in this stage, about 2400 r. Percentage of androgenetic males increases up to about 15,000 r, then gradually decreases until none is produced at about 54,000 r which is the lethal dose for the cytoplasm in this stage. Percentage of androgenetic males can be increased at doses above 15,000 r by fractionation.

Eggs x-rayed in prophase I × untreated sperm

Gynogenetic males develop from the unfertilized eggs. No androgenetic males develop due to absence of type of chromosome aberration necessary for their formation. Dose-hatchability curve for gynogenetic males is exponential up to about 15,000 r, after which it falls at an increased rate. It can be restored to an exponential curve by fractionating dose. Lethal dose is about 54,000 r. This is the lethal dose for cytoplasm. That for the chromosomes in this stage is considerably higher.

Chromosomal vs. cytoplasmic injury

Some androgenetic males survive after dose over twenty times greater than that which is lethal for chromosomes of eggs in which they develop. At all doses they resemble the controls and differ significantly from gynogenetic males in visible mutation rate, viability, and fertility. The changes peculiar to gynogenetic males must be chromosomal in origin since both types of males develop in irradiated cytoplasm. Evidence suggests that these changes are directly induced and supports the target theory of chromosomal injury.

Since there is no evidence for chromosomal injury in surviving androgenetic males, the reduction of their number at doses above 15,000 r, through embryonic death, must be directly cytoplasmic.

The increase in survival of both gynogenetic and androgenetic males in response to fractionation of dose must be due to reduction of cytoplasmic injury since they have only x-rayed cytoplasm in common.

CONCLUSION

At doses from 50 r to about 15,000 r, death of Habrobracon eggs (one stage more sensitive than sperm, the other less so) is due to chromosomal injury. It is not reduced by fractionation of dose or changes in intensity.

Above 15,000 r, x-rays induce cytoplasmic injury which may exert a lethal effect on developing embryos. It is reduced or prevented by fractionation of dose.

Injured cytoplasm has an "all or none" effect. It may kill embryos but does not induce visible mutations in untreated chromosomes or reduce fertility or viability of survivors. Its expression resembles, therefore, dominant lethal genetic effects and it acts directly in killing the embryo and not indirectly through injury to untreated chromosomes.

Evidence supports the target theory of chromosomal injury.

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PROGRAM AND ABSTRACTS OF SEMINAR PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1949

July 12

Plus and minus mutations in biochemical requirements in Salmonella typhimurium. H. H. Plough, Madelon R. Grimm and Martin L. Vogel, Amherst College.

For more than two years we have been studying biochemical mutations in *Salmonella typhimurium* and this work is being continued at the Marine Biological Laboratory. The work is of interest like that on *Neurospora* and on *Escherchia coli,* because it appears to be uncovering some of the basic biochemical activities of genes. In addition in *Salmonella* the serological or antigenic variants are so well known that it seems possible to attempt some correlated study of biochemical mutants and antigenic variants.

Our method is to expose living organisms in quartz flasks to ultra violet, or at Woods Hole, to X radiation in doses up to 15,000 R units. Then these are diluted and plated according to Lederberg's method on agar containing the essential salts and an energy source, glucose. After incubation the plates are layered with a complete medium, and re-incubated. Small colonies which appear after the second layer are often constant mutant strains which are found to require one or more amino acids or vitamins as essential growth factors.

By this method we have found many mutants of which the most frequent are strains requiring cysteine, proline, histidine and thiamin. One freshly isolated strain required thiamin, and this has mutated to a strain which is thiamin independent. Thus mutations occur in both plus and minus directions. Energy utilization in relation to reduced sulfur in the cysteine-requiring strain is being studied.

Evidence that response to fractionation of x-ray dose in Habrobracon eggs is cytoplasmic. Anna R. Whiting. No abstract submitted.

X-ray mutations and fecundity of Mormoniella. P. W. Whiting and Marion E. Kayhart. No abstract submitted.

July 19

Reversible enzymic reduction of retinene to vitamin A. Alfred F. Bliss.

Fresh solutions of bleached visual purple form vitamin A (Bliss, *Biol. Bull.* 1946). Morton and co-workers have shown that vitamin A is also formed when synthetic retinene (vitamin A aldehyde) is fed or injected into rats. Since vitamin A is an alcohol, and can be dehydrogenated to a typical aldehyde, it seemed possible that the enzyme involved might be the well-known reversible DPN-specific alcohol dehydrogenase. Acetone and ammonium sulfate precipitate of rabbit liver were prepared according to Lutwak-Mann. Bisulfite or cyanide was used as aldehyde trapping reagents to displace the equilibrium towards the aldehyde side. Crystalline vitamin A, dissolved with a detergent, Tween 80, was the substrate and coenzyme I, the hydrogen acceptor. The aldehyde formed was released by dilution or alkaline destruction of the addition compound (absorption maximum ca. 330 mμ), and extracted with petroleum ether. Experiments to date have shown up to 40% conversion to the aldehyde. Complete reversibility of the dehydrogenation is easily accomplished in the presence of enzyme and reduced coenzyme.

Wald has reported that retinene in retinal rods and extracts of whole retinas is irreversibly reduced by the retinene reductase of the rods in the presence of reduced coenzyme I. We have confirmed the activity of isolated intact rods. However, we have found that the reductase activity of extracts of whole retinas is an artefact due to the large amount of enzyme from the non-visual part of the retina. Furthermore, we have found that vitamin A formation by isolated rods is freely reversible in the presence of cyanide.

We therefore need no longer assume a closed visual cycle to explain the formation of retinene from vitamin A. Instead, it is probable that the dehydrogenation is accomplished by alcohol dehydrogenase with the formation of visual purple which acts as the physiological trapping compound for retinene.

Some effects of ultra-violet light on the catalase activity and on photosynthesis of Chlorella pyrenoidosa. A. Frenkel.

It has been demonstrated by Arnold (1933) that ultra-violet light ($\lambda = 2537$ Å) inhibits the light reaction of photosynthesis, as he could show that the percentage inhibition was the same in flashing and in continuous light.

On the assumption that ultra-violet light could also have affected the oxygen liberating catalyst in photosynthesis, the catalase activity of Chlorella cells was tested before and after irradiation with ultra-violet light. In each case an increase in the catalase activity was found (Table 1), a phenomenon which had been observed by Euler (1933) with irradiated yeast cells. No catalase was released by the cells, as the suspending fluid showed no activity after the cells had been centrifuged off.

TABLE I

Measurements were performed with 0.026 cm.^{3} (wet volume) of *Chlorella pyrenoidosa* cells suspended in 3 ml. of 0.035 M KHCO$_3$ and 0.065 M NaHCO$_3$ at 25° C.

Time of exposure to ultra-violet light. Incident intensity 1.3 ergs./mm.^{2} sec.	Photosynthesis in per cent of control	Rate constants (sec.$^{-1}$) of decomposition of H$_2$O$_2$ by 1 cm.^{3} (wet volume) of Chlorella cells
0 minutes	100	1.3
3 minutes	80	1.5
6 minutes	50	2.5
11 minutes	0	9.8

Arnold had observed that the absorption spectrum of chlorophyll does not change after irradiation of Chlorella suspensions by ultra-violet light. We have noticed, however, that in intact cells the transmission of the red chlorophyll peak increased after the ultra-violet light treated cells were exposed to visible light. This bleaching of chlorophyll, for a given dose of ultra-violet light, is a function of the intensity and of the time of exposure to visible light. In some way the energy transferring mechanism in photosynthesis has become uncoupled and the light energy directly or through some photoperoxide produces the bleaching of chlorophyll.

Biochemical properties of succinoxidase from salmonella aertrycke.[1] Ernest Kun.

Succinoxidase was obtained by lyophilization of the washed insoluble residue of the microorganisms, which were previously sterilized at 60° C. three times for twenty-five minutes. This enzyme catalyzed the oxidation of succinate by molecular oxygen. K$_s$ was found to be 2.2.10^{-3} M succinate, heat of activation of succinate oxidation 10.470 cal. per moles of succinate. Cytochrome C inhibited the aerobic oxidation of succinate, the type of inhibition being of competitive nature. Cytochrome C was reduced by the enzyme, which reduction was not influenced by succinate. Since cytochrome oxidase was not found to be present in the preparation, it was assumed that molecular oxygen was activated by a different catalyst than the cytochrome system. Some evidence was obtained that H$_2$O$_2$ is formed during succinate oxidation by way of a flavin-like catalyst. The succinoxidase preparation had catalase, peroxidase, and fumaric hydrogenase activity; the latter not being inhibited by inhibitors which completely stop succinic dehydrogenase activity such as malonate and iodoacetamide. It seems possible that in the course of the preparation of the Salmonella succinoxidase the cytochrome oxidase is denatured and a flavoprotein which is associated with the succinoxidase system can serve as acceptor of molecular oxygen.

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[1] Kun, Ernest, and Abood, L. G.: *J. Biol. Chem.* 180, vol. 2, in press.

An enzymatic product with acetylcholine-like activity, derived from brain extract. DAVID NACHMANSON, S. HESTERIN, H. VORPHAIEFF. No abstract submitted.

JULY 26

Evidence for activity of desoxyribonuclease in nuclear fusion and mitosis by the use of d-usnic acid. ALFRED MARSHAK.

A crystalline substance, d-usnic acid $C_{18}H_{16}O_7$, was isolated from the lichen *Ramalina reticulata.* It was found to inhibit the growth of several species of bacteria, but was especially active against the human tubercle bacillus. It also prevented cleavage and completely inhibited uptake of radioactive phosphorus by the fertilized eggs of Arbacia, but had no effect on their oxygen consumption. It was found that in the presence of usnic acid ($10\,\gamma$/ml., a dose sufficient to completely inhibit cleavage), the sperm penetrated the egg and the sperm nucleus reached the surface of the egg nucleus at the same time as in the controls (8 to 12 minutes after fertilization). However, the normal fusion with the egg nucleus and dispersion of the Feulgen-positive material of the sperm nucleus did not take place. This suggested inhibition of the system desoxyribonucleis acid (DNA)—desoxyribonuclease (DNase). The activity of DNase (crystalline) and DNA isolated by the method of Gulland (N/P equals 1.66) in the presence and absence of sodium usnate was then investigated using viscosity change as index of activity. It was found that $10\,\gamma$ of the usnate could completely inhibit the enzyme action, and that this inhibition required the presence of cobalt ($CoCl_2$). Cobalt in the absence of usnate gave no inhibition of the enzyme.

Unlike streptomycin, usnic acid did not form complexes with DNA so that its action was probably on the enzyme rather than the substrate.

Since usnic acid also inhibited cell division if added to fertilized eggs after the prophase of the first cleavage was initiated, it follows that mechanisms for the dispersion of DNA such as DNase are involved in the mitotic cycle as well as in the fusion of sperm and egg nucleus.

The growth and metamorphosis of the Arbacia punctulata pluteus. ETHEL BROWNE HARVEY.

The well known three- or four-day pluteus of Arbacia with two long anal arms and two short oral arms and bright red pigment spots will develop no further at Woods Hole unless specially fed. The best food is the diatom, *Nitzchia closterium,* which is cultured on Miquel's solution. The later development of the pluteus and metamorphosis is described with photographs. In about ten days after fertilization a new pair of arms with red tips grows out and later another pair of long arms. The body of the adult grows up inside the pluteus. In about two months, the five primitive ambulacral feet appear, and then primitive spines between the ambulacral feet. The animal tumbles about on its arms as well as swims with its cilia. In about two and a half months, after reaching full development, the arms begin to go to pieces, by resorption and by breaking off. The body of the adult grows larger inside the pluteus, and metamorphosis occurs in about four months after fertilization the later stages taking place very rapidly. The young adult is about 0.5 mm. in diameter.

The pluteus from the centrifuged egg develops in the same way. The pluteus from the white half-egg obtained by centrifuging is at first colorless and smaller than that from the whole egg. It acquires the red pigment spots in three or four days, and if fed develops in the same way as the whole egg and is similar in size and pigmentation.

Motion pictures showing the reactions of cells in frog tadpoles to implants of tantalum.[1] CARL C. SPEIDEL.

The reactions of cells in the tail of the frog tadpole to implants of tantalum have been recorded by cine-photomicrography. The same implants have been kept under observation for as long as several months. Motion pictures have been taken at both normal and low speeds. Cell movements are especially well revealed by the low speed picture.

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[1] Aided by a grant from the American Cancer Society (Committee on Growth). The tantalum was supplied by the Ethicon Suture Laboratories, New Brunswick, N. J.

Pictures of implants of tantalum powder show the early responses of leukocytes, endothelium of lymph and blood vessels, epithelium, and fibroblasts. Cells of these types near the implant may pick up tantalum granules. Leukocytes are most active in this respect. Frequently a cutaneous papilla forms after 2 or 3 days at the site of an implant. It grows out into a finger-like structure carrying some of the tantalum implant with it. It becomes eliminated after several more days by pinching off at the base.

Tantalum-laden macrophages often enter lymph or blood vessels and are carried away. Other tantalum-laden macrophages remain at the implant site for long periods (the motion pictures record this up to 84 days). Such cells exhibit continual slow adjustments. Encapsulation of tantalum and tantalum-laden macrophages often occurs after about 12 days, particularly in the case of deeply located implants. In later stages the capsule shell may become somewhat fibrous.

Tantalum wire implants are surrounded during the first day by a thin film of leukocytes. These isolate the wire from the adjacent tissues which display very little inflammation. Short lengths of tantalum wire thus walled off persist indefinitely. The surrounding leukocytes seem to form a fairly complete syncytial shell after a few days.

Effects of temperature upon survival of newborn guinea pigs subjected to anoxia. JAMES A. MILLER. No abstract submitted.

AUGUST 2

Labile P in nucleic acids. ABEL LAJTHA.

There is a striking parallelism between muscle and other organs, for example kidney and liver. If we let rabbit muscle or any other kind of muscle stand at room temperature, the ATP in it is gradually split; and parallel with the decreasing ATP concentration, the elasticity of muscle fibers decreases, a stiffness gradually develops (rigor mortis), and the solubility of the highly viscous muscle protein actomyosin decreases too.

The post mortem changes in kidney are analogous. If we mix fresh minced kidney with strong salt solutions, a greatly viscous extract is obtained, and the sticky solution shows a strong double refraction of flow. If, before extraction of the kidney, we let it stand at room temperature for about half an hour, the viscosity of the solution will be very small, there will be no DRF and it will not appear sticky—showing that only very small amounts of the kidney structure-protein went into solution if any at all.

In the muscle the changes are mostly restored by adding physiological amounts of ATP. Even large amounts of ATP do not restore the lost solubility of structure proteins in kidney. The analysis shows that compared with the muscle, there is only about one tenth as much ATP in the kidney.

The question arises whether in kidney the nucleic acid plays the role played by ATP in muscle. The first question in approaching this problem is whether nucleic acids contain labile P.

Nucleic acids were prepared from kidney and liver in three different ways. In a set of experiments emphasis was laid on purity of the product, in another on quantitative yields, and in the third on mildness of the method avoiding all possibility of hydrolysis.

Working with rabbits the animal was killed by decapitation, the abdomen immediately opened, the organs excised and within three minutes after the death of the animal the organs were in the Waring blender.

To prepare pure nucleic acids the organs were washed with cold trichloracetic acid, then with lipoid solvents, and with strong NaCl, reprecipitated with acids at pH 2.5 several times, and washed with lipoid solvents again several times. As in the other methods followed, the purification was made with pentose and desoxypentose tests and with ultraviolet absorption spectra. With this type of reprecipitation we get pure nucleic acids very fast, and working at a low temperature we can retain almost all labile P.

To get quantitative results I extracted the organs with hot NaCl solution containing 5 per cent Na_2CO_3. Extracting three times for about fifteen minutes the analysis of the remainder showed that about 98 per cent of the P containing compounds were dissolved. Precipitation was made complete with the combined action of acid and alcohol. This method, however, must be corrected, as experiments with nucleic acids prepared in another way and after being boiled in basic NaCl solution showed that about 10 per cent of the labile P is split by 45 minutes boiling in a salt solution containing 5 per cent Na_2CO_3.

To work as fast as possible and retain all labile P groups, the organ was washed with cold alcohol, then with water, and then extracted in many ways. One of the methods used for example was extracting it with hot water. All the extracting solutions were then analyzed for nucleoprotein P and labile P afterwards.

The results of these experiments are that the nucleic acids of kidney and liver contain a labile P which is hydrolyzed in normal acid in 10 minutes and which amounts to about 20 per cent of the total P.

This would show that for approximately every tetranucleotide unit there is one labile P in the nucleic acid.

The accumulation of phosphate and evidence for synthesis of adenosinetriphosphate in the fertilized sea urchin egg.[1] EDWARD L. CHAMBERS AND WILLIAM E. WHITE.[2]

Unfertilized and fertilized eggs of *Strongylocentrotus purpuratus* were prepared in 0.2 per cent suspensions in sea water containing 0.060–0.100 mg. P per 1000 ml., maintained at 15° C. The concentration of inorganic phosphate in the suspension fluid was determined at intervals using the Deniges-Atkins method.

The concentration of inorganic phosphate in the sea water overlying the unfertilized eggs underwent a slight increase, while that overlying the fertilized eggs underwent a marked decrease. Similar results were obtained using the eggs of *S. franciscanus.*

Other experiments, based on the above findings, were done with P^{32} as $P^{32}O_4$ added to a 0.2 per cent suspension of fertilized eggs. The rates of disappearance of P^{32} and of P^{31} from the medium were found to be identical. Also, the rate of uptake of P^{32} by the eggs was found to correspond exactly with the loss of P^{32} from the medium. These data indicate that the rapid uptake of P^{32} by fertilized eggs (Brooks and Chambers, *Biol. Bull.*, 95, 1948) is due to an accumulation of phosphate within the egg while the slow uptake of P^{32} by the unfertilized egg represents an exchange process.

The rate of uptake of phosphate, in the concentration range of 0.015–0.100 mg. P/1000 ml. sea water, was 0.003–0.004 mg. P/ml. fertilized *S. purpuratus* eggs/minute.

The inorganic (IP) and labile phosphorus (LP) in the 5 per cent trichloracetic acid extracts of unfertilized eggs and of fertilized eggs at 70–80 minutes after insemination (50 per cent cleavage time at 15° C. being 110 min.), were determined by the method of Borbiro and Szent-Györgyi (*Biol. Bull.*, 96, 1949). The distribution of P^{32} in the fractions was also determined. Labile phosphorus is defined as the P hydrolyzed in the presence of 1 N HCl at 100° C. in 10 minutes. The non-hydrolyzed P represents that fraction which remains in the TCA extract after extraction of the inorganic and of the labile P with isobutyl alcohol.

The results are presented in the accompanying table.

In the fertilized eggs at 70–90 minutes after insemination there was observed, in the TCA extract, a marked decrease in the inorganic P fraction and an increase of labile P. The increase of labile P was greater than the decrease of inorganic P and was due undoubtedly to penetration into the egg of phosphate from outside. The labile phosphorus has been identified as P split from adenosinetriphosphate (White and Chambers, Revue de pathologie comparée et d'hygiène générale. In press).

In the unfertilized and fertilized eggs 95–96 per cent of the total P^{32} was found in the acid-soluble extracts. As seen in the table 86 per cent of the P^{32} in the acid soluble extract was found in the LP fraction of the fertilized egg, indicating that the major portion of the phosphate which entered the eggs was incorporated in adenosinetriphosphate. These results indicate that,

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[1] Aided by a grant from the N.C.I., U.S.P.H.S.

[2] University of California, Berkeley, New York University, and the Eli Lilly Research Laboratories, M.B.L., Woods Hole, Mass.

Distribution of P and P^{32} in the acid-soluble extracts of unfertilized and fertilized eggs

	Mg. P/Ml. eggs			% Total P^{32} in acid-soluble extract			Relative specific activity		
	IP	LP	IP + LP	IP	LP	Non-hydrolyzed P	IP	LP	Non-hydrolyzed P
Unfertilized	.059 mg.	.410 mg.	.469 mg.	18%	73%	8%	1.0	.49	(.08)
Fertilized	.028 mg.	.451 mg.	.479 mg.	8%	86%	6%	1.0	.65	(.07)

following fertilization, a synthesis of ATP occurs at the expense of inorganic P. The decrease in inorganic P is accompanied by the entrance of P from the external medium.

Some methods of producing traveling contraction nodes in adult frog skeletal muscle fibers (motion picture). B. A. COOKSON [1] AND FLOYD WIERCINSKI.

Small bundles of muscle fibers, teased from the adductor magnus of *Rana pipiens,* were immersed in a solution containing 1.3 per cent NaCl and approximately 0.7 volume per cent H_2O_2. After approximately 2 minutes small contraction nodes were seen forming in various regions of the fibers. These nodes usually recurred in the same regions at regular intervals. After originating, they traveled along the fibers. Usually the node as it formed split into two nodes traveling in opposite directions. Frequently the nodes collided and canceled out. Sometimes contraction nodes were formed which involved only half the circumference of the muscle fiber.

Reduction of the NaCl concentration to .65 per cent (approximately isotonic with frog's blood) resulted in an absence of response. The response could be restored by increasing the osmotic pressure through the addition of non-electrolytes such as 1.5 per cent (by volume) glycerol or 7 per cent sucrose. The response was not lost when 1.6 per cent KCl was substituted for 1.3 per cent NaCl.

A slight increase in the H_2O_2 concentration produced, instead of traveling contraction nodes, large stationary areas of fiber which rhythmically contracted and relaxed. During the contraction the sarcolemma became wrinkled. A slight decrease in the H_2O_2 concentration resulted in a complete absence of response. Approximately .002 per cent 2-methyl naphthoquinone (a powerful anti-choline acetylase) could be substituted for 0.7 volumes per cent H_2O_2.

Certain concentrations of 2-methyl naphthoquinone in .65 NaCl produced asynchronous contractions of the myofibrils. Upon making the NaCl solutions hypertonic these asynchronous contractions became first more vigorous and then synchronized as the usual traveling contraction nodes involving the whole fiber.

A solution containing 1.3 per cent NaCl, 0.75 volumes per cent H_2O_2, 1 per cent glutathione and enough 0.1 N NaOH to adjust the pH to 7.4 was found to keep its potency for over 2 days, whereas the solutions containing only 1.3 per cent NaCl and 0.7 volumes per cent H_2O_2, due to the instability of the H_2O_2, remained potent for only 2 to 3 hours. With the former solution it was possible to produce traveling contraction nodes in isolated fibers with injured ends.

Investigation on muscle fibers. ANDREW G. SZENT-GYÖRGYI.

The material used was the psoas muscle washed with glycerol as described by Dr. A. Szent-Györgyi (*Biological Bulletin,* 96, 140, 1949). The question was how far the behavior of these fibers corresponds to that of actomyosin threads. The actomyosin thread contracts, in the presence of ATP at low salt concentrations up to 0.2 M NaCl or KCl. At higher salt concentrations it dissolves and dissociates into actin and myosin. The contraction of the glycerinated fibers is independent of salt concentration. They contract maximally even at 0.5 M NaCl, the highest salt concentration used. The close packing and the prolonged washing in glycerol stabilize the actomyosin in the fibers so strongly that ATP cannot dissociate it even at high salt concentrations.

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[1] This work was done while one of us (B. A. C.) was holding a Cancer Fellowship of the National Institute of Health, U. S. Public Health Service.

If the structure is loosened by high concentration of ions which have specific dissociating action (sodium pyrophosphate, $NaHCO_3$, NaOCN) for a few minutes, the white, opaque and brittle muscle becomes transparent and slightly elastic. The contraction is maximal between 0.1 and 0.25, M NaCl. Below and above this salt range there is no contraction, the difference being very sharp. The fibers behave after the above treatment like the actomyosin thread.

The effect of the treatment can be reversed by immersing the muscle into 0.1 M NaCl for a few minutes.

The results indicate that the contraction of the pyrophosphate treated muscle takes place in at least two steps. The first is actomyosin formation, which depends on salt concentration. The second is the contraction itself, caused by the ATP only after actomyosin was formed.

On the structure of fibrin clots. ELEMER MIHÁLYI.

Several investigators of the late nineteenth century reported the solubility of fibrin clots in urea solutions. Wöhlisch and his co-workers, however, could not confirm this finding.

The problem has considerable importance because the protein gels and coagula, where the particles are bound by secondary forces, are all soluble in urea solutions. Insolubility may thus be an indicator of co-valent bonds between the fibrin molecules.

In the experiments which will be described, the solubility of fibrin in concentrated urea solutions was definitely confirmed. When urea is dialysed out, the clot is reconstituted. The pH of the fibrin solution during the dialysis decides whether a coarse or a fine type gel will be formed.

The viscosity of fibrin in 30 per cent urea solution is normal and equal to that of fibrinogen in similar conditions. The pH has no influence on the viscosity. At 20 per cent urea concentration the viscosity is increased by increasing the pH up to 8.6. Further increase of pH again decreases the viscosity. At still lower urea concentrations the increase of viscosity on alkalinisation leads to gelification.

The solubility of fibrin in urea solutions makes it possible to investigate in Tiselius apparatus the electrophoretic mobility of this protein. If the action of thrombin involves some of the ionising groups of fibrinogen, a study of the differences in electrophoretic mobilities between fibrinogen and fibrin may give some information on the nature of the process of clotting.

It was found that fibrin has a lower mobility above and a higher mobility below its isoelectric point than fibrinogen. The isoelectric point of the two proteins are very close together. In 20 per cent urea solution fibrinogen is isoelectric at pH 5.5, fibrin at 5.6. The results indicate that in the zone alkaline to the isoelectric point the net charge of fibrin is lower than that of fibrinogen.

AUGUST 9

Hemolytic action of anionic detergents. LOIS H. LOVE.

The anionic detergent, sodium dodecyl sulfate, acts as both a hemolytic and anti-hemolytic agent. The course of hemolysis is complex, being very rapid for a few seconds and then stopping or slowing greatly, often for many minutes, before the remaining cells hemolyze. The cells which survive the initial, rapid hemolysis can be made to hemolyze rapidly by procedures which would be expected to remove detergent from the cells (dilution or the addition of $BaCl_2$).

The effect of temperature reflects this complexity. For each detergent concentration there is a temperature at which hemoylsis is slowest. The effect is so large that an increase of 1° C. can change the hemolysis time from less than 1 minute to more than 1 hour.

The pH effect depends on the detergent concentration. With high concentration the hemolysis time decreases from pH 6.0 to 8.0. With low concentrations the order is reversed. The results with temperature and pH appear to be due to different effects on the hemolytic and protective stages of hemolysis.

Sodium tetradecyl sulfate acts like sodium dodecyl sulfate if complications due to its low solubility are avoided. It is also shown that the presence of micelles can alter the course of hemolysis.

How simple are the so-called "simple hemolysins"? M. H. JACOBS, CAROLYN M. STOUT, MARIAN W. LEFEVRE AND W. E. LOVE.

Included among the so-called "simple hemolysins" are substances like soaps, bile salts, and saponin the behavior of which is so complex as to cause difficulty in explaining and frequently in repeating experimental results. Further evidence of this complexity is the appearance in the literature of terms such as "catastoichic," "zone phenomenon," etc. to describe behavior that has not been satisfactorily explained.

A clue to some of the complexities of behavior of the substances in question is provided by a study of a truly simple hemolysin, butyl alcohol, which we have previously shown (*Biol. Bull.*, **77**, 319, 1938; **93**, 223, 1947) to be capable of producing a condition of extreme and irreversible cation permeability that leads to swelling, rather than to the normal shrinkage, of erythrocytes at alkaline reactions. Like butyl alcohol, sodium oleate can also be shown to produce a condition of cation permeability. In the frequently discussed system: erythrocytes, oleate and alkali, the factor that determines whether the alkali shall be protective or destructive is not whether it is added before or after the oleate, but rather whether it is added before or after the cells have been made cation permeable by the oleate.

Complicating and sometimes obscuring the action of this factor is another to which we have recently directed attention (*Federation Proc.*, **8**, 80, 1949), namely, the antihemolytic effect of agents like soaps and bile salts, which by forming a layer at the surface of the erythrocyte prevent the escape of hemoglobin under conditions where it would otherwise occur. The relation of this type of action to the "zone phenomenon" is obvious. In addition to these two factors, several others now under investigation further complicate the situation, but appear to be no less capable of accurate analysis than those here mentioned.

An analysis of the photoelectric method of measuring permeability.[1] F. R. HUNTER.[2]

In an attempt to avoid some of the difficulties of interpreting data obtained using the hemolysis or the swelling technique, an analysis was made of Wilbrandt's shrinking technique. This consists of equilibrating erythrocytes in a solution of non-electrolyte in Ringer Locke. An aliquot of cells containing the non-electrolyte is then transferred to a salt solution and the rate of shrinking as the non-electrolyte leaves the cells is measured photoelectrically. Series of shrinking curves can be obtained by using non-electrolyte solutions of various concentrations and by measuring shrinking in salt solutions of different concentrations. As would be expected, a greater volume change is obtained by increasing the concentration of non-electrolyte or decreasing the concentration of the salt solution in which the cells shrink. Hematocrit determinations showed that chicken erythrocytes behave as perfect osmometers (using a b value of 0.355 which is the dry weight) when placed in salt solutions of tonicities between R.L. and 2 × R.L. The galvanometer deflections of the apparatus are linearly related to volume changes over this same range of salt concentrations. There is some deviation of observed from calculated volume changes of cells equilibrated in the non-electrolyte-R.L. solution and allowed to shrink in a salt solution. Spectrophotometric data show that this deviation is due in part to slight, initial hemolysis. However, since this hemolysis occurs initially, rather than terminally as it does when swelling measurements are made, the shrinking data are more easily interpreted and constancy of time for one half the galvanometer was obtained from day to day. It is believed that if chicken erythrocytes are equilibrated in 0.6 M non-electrolyte in R.L. and allowed to shrink in 1.875–1.625 × R.L. solutions, a change in time for one half the total deflection is an indication of a permeability change.

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[1] This work was supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and the Faculty Research Fund, the University of Oklahoma.

[2] Department of Zoological Sciences, the University of Oklahoma, Norman.

Potassium and sodium exchange in rabbit erythrocytes treated with butyl alcohol. A. K. Parpart and J. W. Green. No abstract submitted.

August 16

Studies on degenerating testicular cells in immature mammals. I. Analysis of degeneration in primordial male germ cells and in a hitherto undescribed germ cell in albino rats aged one to nine days. Ezra Allen.

In the early postnatal albino rat testis three waves of degenerating cells have been reported. The first involves the so-called "primordial" germ cells. These are found in the central part of the testis cords. They are quite abundant in the 1-day testis, gradually decrease in number till very few if any remain at 9 days of age. The second and third waves of degeneration take place with the development of the spermatocytes and the spermatids. (Hoven, 1914; Firket, 1920; Hargitt, 1926.) The details of the degenerative process of the primordials are described in this paper as well as the degeneration of another type of cell called a "large" cell.

I. Degeneration of the primordial cells. Shortly before birth these cells become vacuolated. At birth their cytoplasm is still vacuolated. Vacuolation proceeds until none remains, and the cell wall has disappeared. Meanwhile the chromatin breaks up into small particles which persist as the nucleus decreases in size. These are described as pycnotic cells. At the age of 7 days the pycnotic cells have practically replaced the primordial complete cells. Gradually the pycnotic cells lose their chromatin and finally are absorbed. A few remain at the 9th day of age.

II. The "large" cells. The large cells differentiate from the basal cells of the sex cords, beginning to appear at 3 days. At metaphase they are about the size of the full-sized primordial cells. Their chromosomes are large and well defined. Cell division may occur normally, or at meta- or anaphase the chromosomes may break up into irregular large and small bodies which lie in a dense mass of achromatic substance. These cells become two to three times the diameter of their normal state at metaphase, and are spoken of as "giant" cells. Their chromatin bodies become progressively smaller and finally are lost to view in a delicate matrix which resembles the matrix of the central part of the sex cord; absorption follows. Most of them have been absorbed at 9 days. The large cells are not very numerous. A few have been found with close to the haploid number of chromosomes. One showed clearly either 21 or 23 chromosomes. Generally in the giant cells the number was close to or at the diploid.

Comparative study of lipids in fish. Charles G. Wilber.

A review of the pertinent literature indicates that little information is available concerning the amounts and the types of lipids present in fish, especially Arctic aquatic forms. Specimens of *Pygosteus pungitius* were collected in Arctic Alaska during the summer of 1949. They were analyzed for total fatty acids, cholesterol, and phospholipid. Similar analyses were made on 2 groups of guppy (*Girardinus guppyi*). The mean values for the Arctic fish in per cent fresh tissue are: fatty acid, 4.88; cholesterol, 0.32; phospholipid, 1.87. For the guppies these values are: 8.87, 0.56, and 2.31 respectively. It is obvious that there is more cholesterol and fatty acids in the guppies than in *Pygosteus*. Statistically, the phospholipids in either fish are the same. Ratios of the various lipids in the different fish were calculated with the following results: there is more phospholipid in relation to cholesterol or to fatty acids in the Arctic fish than in the guppy. This fact may be interpreted as indicating a higher level of fat turnover in the former than in the latter. The ratio of cholesterol to lipid phosphorus is lower in the stickleback than in the guppy. This low ratio indicates a decreased amount of water in the tissues of the Arctic fish, a fact which may be correlated with the resistance of the Arctic fish to freezing injuries.

The structure of insulin and the cyclol hypothesis. Dorothy Wrinch.

The x-ray diffraction study of wet crystalline insulin (Crowfoot and Riley, *Nature* **141**: 521, 1938) yields many intensities. If the phases were known, which they are not, the atomic pattern of the crystal could be directly calculated. However, from the Fourier transform of the observed intensities, a precisely equivalent "experimental" vector map can be calculated. There is therefore a second way to use such data as clues to protein structure, if we can interpret the language of vector space. Accordingly x-ray data enable us to assess the validity of the cyclol or any other hypothesis of protein structure, if, but only if, we can discover the characteristic features of the "synthetic" intensity or vector maps (Wrinch, *J. Chem. Phys.* **16**: 1007, 1948) expected from the given hypothesis.

Last year it was reported that all cyclol cages have intensity maxima at ca. 10–11½A (*Biol. Bull.* **95**: 272, 1948). This established a contact between the cyclol hypothesis and the intensity data for many proteins. In the present note a further contact is claimed between the hypothesis and the published section of the experimental vector map of wet crystalline insulin (Crowfoot, *Russian Chem. Jour.* G, 15, 215, 1946).

We may visualize wet crystalline insulin as a three-dimensional network of molecules, arrange in particles, in a water medium. It is inconvenient and unnecessary to construct the corresponding vector map because of the many water entries. Instead we reduce the entire crystal to the water level, replacing the water by zero entries and the protein molecules by *reduced* molecules. The resulting vector map is then the true vector map reduced to the corresponding level. To obtain it, we enter the vector maps of each reduced molecule on itself at the origin, the vector maps of each on every other at appropriate points. There results a map which for a certain domain about the origin represents simply the superpositions of self-interactions of each reduced molecule, the rest of the map being predominantly a highly complex manifestation of the molecular pattern of the particles, from which no information regarding the structure of the molecules can be obtained.

Given a C_1 cage skeleton, or twin skeletons on cube or tetrahedral faces, of density d, with emergent R-groups of density d_r and interior of density d_i, reduced to the water level d_0, it will be shown elsewhere that the gross structure is manifested in characteristic manners in vector space. When d is larger than d_0 and d_r, d_i are sufficiently smaller than d_0, the central trigonal section has a quasi-hexagonal *hill* region about the origin, enclosing and enclosed by *valley* regions. If a is the average of C—C and C—N bond lengths in the cyclol skeleton, say 1.5 A, the edge of the outer hexagonal boundary of the hill region is ca. $4a\sqrt{6} \sim 14.7$ A.

The corresponding section of the experimental vector map of wet crystalline insulin (Crowfoot, loc. cit.) also has about the origin, a *hill* region, approximating a hexagon, with outer edges within an angstrom or less of 14.7 A. Furthermore this region likewise encloses and is enclosed by *valley* regions. The direct relationship between the two maps is confined to this valley-hill-valley region about the origin. However, it is this region alone which gives information as to the gross structure of the molecules in the insulin particle.

It is therefore claimed that contact is now established between certain typographical features of the published section of the experimental vector map and certain topographical features of the corresponding section of the synthetic vector map derived from a molecular skeleton or twin skeletons having the size and shape of the C_1 type of structure proposed for the skeletons of the molecules of insulin.

(This work is supported by the Office of Naval Research under contract N8onr-579.)

The development of Menidia-Fundulus hybrids. J. M. Moulton. No abstract submitted.

GENERAL MEETINGS

Development of spermatozoa in albino rat from 9 to 50 days of age. Ezra Allen.

The age of 9 days marks the stage when the primordial germ cells have practically all been absorbed. There remain only the indifferent basal cells which give rise to several generations of cells by meiosis, terminating with the mature sperm. Some degeneration accompanies the process.

I. Differentiation of the sperm. The first step in the transformation of the basal cells is a well-marked change in their appearance. At 12 days the chromatin gathers into rather large unequal masses, in preparation for mitosis, which occurs at 13 days. The daughter cells become leptotene cells. By 14 days, leptotene cells form; at 15 days they become abundant. Pairing and splitting of chromosomes follow. By 18 days or a little earlier the pachytene stage is reached. No further differentiation takes place for about 8 days. The epithelium increases in number of layers and the pachytene cells increase proportionately. At 26 or 27 days metaphase is reached; at 39 days sperm is present in some tubules; at 42 days in nearly all tubules, but none in the lumens. At 48 days they have matured and are passing into the epididymis.

II. Degeneration phenomena. Degeneration is accomplished in a number of ways. 1. A tubule at any age between 14 days and maturity may lose all of its basal cells except the outermost layer, which usually does not degenerate. 2. Up to 44 days, exfoliation into the lumen of normal or degenerate cells. 3. At 14 days, tubules which lie next to the rete may discharge their cells into the rete. 4. Several types of degenerate cells may form in the epithelium. (a) Cells with a few rather large pycnotic bodies. (b) Cells with a large number of tiny pycnotic bodies. (c) Cells with a large number of dense granules surrounded by dense cytoplasm. (d) These pass into a small cell with structureless matrix. (e) Vacuolation of different types of germ cells. (f) Multinucleate giant cells.

On the source of birefringence within the striated muscle fiber. William R. Amberson, R. Dale Smith, Betty Chinn, Sylvia Himmelfarb, and John Metcalf.

Matoltsy and Gerendas (*Hungarica Acta Physiologica* **1**: 116, 1948) claim that in rat muscle the I bands within the sarcomere contain a negatively birefringent "N"-protein which compensates for the positive birefringence of actomyosin filaments, rendering the I-bands isotropic. Similarly, Dempsey, Wislocki and Singer (*Anat. Rec.* **96**: 221, 1946) believe that negatively birefringent phospholipins in the I-bands render them isotropic. These authors used conventional paraffin technic. Barer (*Biol. Rev.* **23**: 159, 1948) discusses the validity of such theories.

It has been shown by us (*Fed. Proc.* **7**: 2, 1948) that myogen and myosin may be extracted from whole rabbit muscles using pyrophosphate to dissolve myosin without removal of actin. We have now applied K pyrophosphate solutions (5 to 10 per cent) to whole muscles moving the telson of Limulus. The relaxed sarcomeres are 8 to 10 micra long. After removal of divalent cations, pyrophosphate solutions are applied, at 0° C. and $pH = 10.5$. The A-band birefringence disappears in from one to four days. Histological detail is well preserved with Z-lines and myofibrils still visible. In the extracts a viscous protein appears, exhibiting flow birefringence. Presumably a protein, responsible for the normal birefringence, has been removed from its loci in the A-band, in or on the myofibrils.

In these extractions we have never observed the development of negatively birefringent areas, so long as the tissues are handled in aqueous solutions. In paraffin sections we have occasionally seen zones of negative birefringence, often in the I-band, but we consider this phenomenon to be an artefact.

Tiselius analysis shows complicated electrophoretic patterns in pyrophosphate extracts. These patterns differ greatly from those obtained from muscles extracted with phosphate buffers at $pH = 8.1$, in which birefringence persists. It now appears possible to derive two different protein mixtures from these muscles, only one of which contains the protein responsible for birefringence.

Echinochrome: its absorption spectra; pK_1' value; and concentration in the eggs, amoebocytes and test of Arbacia Punctulata. Eric G. Ball and Octavia Cooper.

The absorption spectra of crystalline echinochrome ($C_{12}H_{10}O_7$) obtained from arbacia eggs as described by Ball (*J. Biol. Chem.* **114**, VI, 1936) has been determined at pH values from 1.1 to 8.5. From the data obtained the pK_1' value of the pigment at 26° C. has been calculated to be 6.38. The acid form shows three peaks located at $\lambda 255$, 335, and 475 mμ with molar extinction coefficients of 1.93×10^4, 0.87×10^4, and 0.65×10^4 respectively. The ionized form also displays three peaks, but centered at $\lambda 275$, 400, and 475 mμ with ϵ values of 2.11×10^4, 0.88×10^4, and 1.12×10^4 respectively. Instability of the pigment first becomes evident spectroscopically at pH 6.8 and increases rapidly as the pH is raised. Crystalline pigment obtained from the tests has the same properties as the egg pigment.

Fresh aqueous extracts of eggs or the amoebocytes of the body fluid of either males or females yield absorption spectra similar to those of crystalline echinochrome. Though the pigment in such extracts is largely bound to protein, it shows the same shift in its absorption spectrum with pH changes as observed for the crystalline pigment. This would seem to indicate that the ionizable hydroxyl group of the pigment is not involved in linkage to the protein carrier.

Acid alcohol extracts of eggs, amoebocytes, spines, or 2 to 2½ day old plutei (obtained through the courtesy of Dr. E. B. Harvey) yield the same absorption spectrum, indicating that the pigment from these different sources is identical. Eggs contain on the average 0.58 g. of pigment per 100 cc. of eggs packed by centrifuging. The amoebocytes, whether from males or females contain 3.78 g. per 100 cc. of packed volume of body cells. The test contains 0.19 g. of echinochrome per 100 g. The average female arbacia can thus be calculated to contain a total of 38 mg. of echinochrome; the male has about half this amount.

Effect of ultraviolet radiation on the rate of cell-division of Arbacia eggs. H. F. Blum, J. P. Price, J. C. Robinson, and G. M. Loos.

Appropriate dosage with ultraviolet radiation (wavelengths ~2700Å to 3130Å) slows the rate of cell-division in *Arbacia* eggs, without permanent damage. Recovery of normal rate is gradual and complete. Under comparable conditions the rate of recovery is about the same if the ultraviolet radiation is applied before fertilization, between fertilization and first cleavage, or between first and second cleavages. Application of ultraviolet within about 20 minutes prior to a given cleavage fails to delay that cleavage, but does delay subsequent ones. This tends to obscure the smooth character of the recovery process, which goes on whether cell-division takes place or not.

Recovery is markedly accelerated by illumination with "visible" light (principally blue-violet, ~4000Å to 5000Å), after dosage with ultraviolet. The white halves of centrifuged eggs exhibit the same recovery phenomenon. Visible light does not enhance recovery if applied before dosage with ultraviolet, nor does it accelerate the rate of cell-division of normal eggs.

Except that visible light does not enhance the recovery, similar results are obtained when x-rays are applied to the eggs in appropriate dosage. This important exception indicates, however, a fundamental difference in the mechanism of action of ultraviolet radiation from that of x-rays.

The tolerance of stenohaline forms to diluted sea water. Marie Boyle and Maxwell S. Doty.

T. A. Stephenson[1] has pointed out that at Nanaimo, British Columbia, there is an abundance of normally open ocean forms in waters of estuarine concentrations. To test the hypothesis that coldness of the water resulted in a tolerance of the organisms to such dilutions several species of algae were exposed, in lots of 15, to 0.8 times the normal concentration of sea water and to the normal concentration (about 33%) at various temperatures ranging from 6° C. to 31° C. When the experiments were over the total injury at elevated temperatures was greater in the case of thalli exposed to dilute sea water than in the case of those exposed to normal sea water. At low temperatures there was either no injury or the injury was considered to be the same for both the thalli in diluted and normal sea water. Thus since organisms such as *Sphaerotrichia divaricata* (C. Ag.) Kylin, sporelings of *Fucus vesiculosus* L., and others can tolerate dilution better in colder water an explanation may be provided for the occurrence of stenohaline forms in some brackish waters.

Reversal of polarity in limbs of urodele larvae. Elmer G. Butler.

The polarity of a urodele limb can be reversed by a method of transplantation so that the original proximal end of the limb becomes the free end of the transplant. Regeneration of such a reversed limb will take place. The operative procedure of reversal consists in the insertion of the distal tip of a limb, after amputation of the hand, into a pocket on the body wall just posterior to the shoulder. After initial healing into the pocket has taken place, the limb is then amputated slightly below the head of the humerus, thus permitting the original proximal region of the upper arm to swing free as the distal end of the transplant. Larvae of *Amblystoma opacum* and *Amblystoma punctatum,* 20 mm. to 45 mm. in length have been used. Fifty-two regenerates have been obtained and studied; forty-one other cases have been fixed for study of histological changes preceding regeneration; fifteen transplants underwent extensive regression.

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[1] 1948. Report on work done in North America during 1947–1948. Privately printed in Grt. Brit. by Neill & Co., Ltd., Edinburgh.

Following the amputation through the upper arm, regression of the transplant begins distally and continues usually until the elbow joint is reached. Then, either a blastema is established and regeneration proceeds, or the transplant assumes the character of a non-regenerating appendage. In the latter case amputation of the tip of the transplant, in particular the removal of a remnant of the humerus at the original elbow level, results in the initiation of regenerative activity.

Within the regenerate, terminal portions of radius and ulna are formed carpals, metacarpals and phalanges are established resulting, in many cases in the development of a well formed hand with four digits. These experiments demonstrate clearly, therefore, that the original proximal end of a forearm, when it becomes the free end of a reversed limb, possesses the capacity for the establishment of a blastema in which are organized the normal components of the distal structures of a limb.

Rhythmic alterations in certain properties of the fertilized Arbacia egg. ROBERT CHAMBERS, EDWARD L. CHAMBERS, AND LAWRENCE M. LEONARD.[1]

The experiments were done on eggs which had been denuded of their extraneous coats, viz., the fertilization membrane and the so-called hyaline plasma layer. The removal of these coats was brought about by placing the eggs, 1½ minutes after insemination, into 0.95 M urea for 1 minute and then transferring the eggs to a mixture of NaCl (18 parts) and KCl (2 parts) isotonic with sea water. In this mixture the eggs develop and undergo cleavage at about the same time as in sea water.

As is well known, the cytoplasm of the fertilized egg undergoes successive changes of its structural and physical framework which cannot be considered simply in terms of changes in overall viscosity. The changes are: (1) the formation within 6 to 8 minutes of a gelated cortex in which the pigment vacuoles collect as they migrate peripherad from all parts of the interior; (2) the development of the growing aster at 10 to 15 minutes accompanied by an extending gelation, which finally involves all of the cytoplasm except for the central "lake," the radial canals and the periphery of the aster beneath the cortex; (3) an interphase during which the cytoplasm is fluid and contains the diminutive nuclear spindle; and (4) shortly before cleavage, the growth of the amphiaster which constitutes a spreading gelation around the two polar regions of the spindle.

The experimental procedures consisted of (1) compressing the eggs in a hanging drop between the overlying slide and underneath a strip of coverslip fastened on the upturned shaft of a coarse microneedle, (2) rupturing the eggs either by tearing with microneedles or by applying a drop of high surface tension inert oil which induced a rupture to permit outflow of the interior, and (3) inducing swelling by immersing the eggs in a 50 per cent solution of the NaCl/KCl mixture.

The following results were obtained: 1. Compression of the eggs, irrespective of the stage of development, resulted in a flattening to 2 or 3 times the original diameter without causing rupture. The flattening process induced disappearance of the asters and caused breaks in the gelated cortex which solated without a rupture of the protoplasmic surface film. It is significant that when the eggs were beginning to cleave a protoplasmic film separated away from the contracting gelated band of cortical material at the furrow. The constricting band persisted for a time after the egg had been converted into a very much flattened disc.

2. Sudden rupture of the egg induced disappearance of the asters but in this case a difference was noted between the astral phases and the interphase. When ruptured during the astral phase the resulting sol state was such that the granular contents of the eggs flowed out rapidly. The

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[1] Under grant of the NCI, USPHS; New York University and the Eli Lilly Research Laboratories, M.B.L., Woods Hole, Mass.

cortex of the egg remained gelated and shrank into a diminutive ball of condensed pigment vacuoles. When ruptured during the interphase there was little or no outflow. Evidently, during this period, the cytoplasm maintains a viscosity sufficiently high to prevent an outflow whereupon the exposed cytoplasmic vacuoles undergo agglutination.

3. Exposing the eggs to hypotonic solutions give indications that the swelling is not accompanied by the disappearance of the asters. During the period of the gelated monaster and amphiaster phases the eggs swelled relatively slowly (determined by serially timed photographs) and 80 to 100 per cent of the eggs burst within 5 minutes. Of significance is the fact that during the late amphiaster when the cleavage furrow was forming the bursting occurred at the furrow where the cortex is at the maximum of stiffness. On the other hand, during the early fluid phase (within 4 to 5 minutes after insemination) the swollen eggs did not burst. More detailed studies were made on the later fluid phase, i.e., the interphase between the monaster and amphiaster stages. During this interphase the swelling occurred relatively rapidly and to larger proportions, but the eggs did not burst even after prolonged exposure.

Evidently the physical state of the egg interior and particularly that of the cortex conditions the bursting in the hypotonic solution.

Theobromine and theophylline effects upon rate and form of Arbacia development. RALPH HOLT CHENEY.

Dimethylated dioxypurines differing only in the position of a CH_3 group were used. Gametes were shed into dishes with different concentrations of the drug-in-sea-water for 15 minutes, then mixed for fertilization. Developmental rate and form in SW and TbSW or TpSW were compared with controls. Experimental molarities included one concentration of approximately maximum solubility. Observations were made at intervals during a three-day period.

All eggs per experiment were shed by one female and all sperm from a single male. Four combinations of untreated and treated gametes were utilized in mixing for fertilization. The combinations were as follows: N ♀ × N ♂, N ♀ × Tb ♂ or Tp ♂, Tb ♀ or Tp ♀ × N ♂, Tb ♀ or Tp ♀ × Tb ♂ or Tp ♂, all mixed and developed in SW. The last two combinations were also mixed and developed in TbSW or TpSW.

Results demonstrate that the immersion of the gametes for 15 minutes prior to mixing did not render the eggs non-fertilizable and subsequently did not destroy the ability of the sperm to fertilize. Evidence suggests that pretreatment of both gametes in theobromine did not have any significant effect since development in SW was equivalent to controls. Gametes pretreated with theophylline, however, were affected, at least by higher concentrations; since, although developed in SW, the fertilized eggs showed definite retardation and failed to develop plutei.

Comparison of the effects of equivalent molarities indicate that Tb is more effective than Tp in retarding the rate of Arbacia development. Both retarded the rate primarily during the gastrula-prism-pluteus sequence. Effects are directly proportional to the concentration of the drug. Comparison with the trimethylated dioxypurine, caffeine, as shown by the author in 1948 (*Biol. Bull.* **94**: No. 1, 16–24), indicates the order of the decreasing effectiveness of these compounds upon the developmental rate and form of *Arbacia punctulata* is theobromine, caffeine, and theophylline.

Inhibition of cleavage in Arbacia eggs and of phosphorylation in cell-free egg extracts by nitro- and halo-phenols. G. H. A. CLOWES, A. K. KELTCH, C. F. STRITTMATTER AND C. P. WALTERS.

The effects of a number of substituted phenols on oxidative phosphorylation by the cell-free particulate enzyme system of Arbacia described in the previous abstract have been measured. Typical results are shown in the table; α-ketoglutarate was used as substrate. Oxygen consumption (c.mm.) and phosphorus esterified (micrograms) are per flask (equivalent to 0.1 ml. eggs) per hour. Negative values for phosphorus esterification denote a net increase in inorganic phosphorus.

The effects previously reported for the living eggs (Clowes and Krahl, *J. Gen. Physiol.* **20**: 145, 173, 1936) parallel those upon the cell-free system in the following respects: The phenols which stimulate respiration and block cleavage also increase oxygen use and block phosphorylation by the cell-free system; dinitrothymol blocks cleavage and phosphorylation but reduces oxygen consumption in both systems; o-nitrophenol and picric acid are inactive in both systems at concentrations up to 0.001 M; the total concentration of each active agent required in the cell-free system (pH 6.9) is smaller than that required to block cleavage in sea water (pH 7.9) and the concentration of the undissociated acid form of each nitrophenol required to block cleavage is virtually identical with that required to block phosphorylation.

These results suggest that the Arbacia egg derives energy for cleavage from oxidative phosphorylation and that substituted phenols block cleavage by interfering with generation of high-energy phosphate bonds.

The authors thank Dr. M. E. Krahl for advice.

Conc. reagent	2,4-dinitro-phenol		4,6-dinitro-cresol		4,6-dinitro-carvacrol		2,4-dinitro-thymol		2,4,5-trichloro-phenol	
	O_2 use	P ester.	O_2 use	P ester.	O_2 use	P ester.	O_2 use	P ester.	O_2 use	P ester.
Moles per 1. × 10^6										
None	36	126	34	131	28	104	38	122	31	102
0.13	36	117	34	119	28	93	34	67	—	—
0.25	—	—	34	112	30	87	24	1	32	107
0.5	39	117	38	68	32	67	25	−22	34	104
1	43	108	42	54	32	32	21	−30	36	104
2	42	77	40	−9	30	−19	21	−34	34	99
4	39	39	34	−22	26	−21	20	−30	36	77
8	—	—	36	−8	30	−17	—	—	37	9
16	38	−4	31	−4	26	−20	—	—	32	−18
32	—	—	—	—	—	—	—	—	30	−17

Toxicity responses of dividing nuclei of Allium as demonstrated with the Sudan black B technique. Isadore Cohen.

The responses of Allium roots correlated with their mitotic behavior has been suggested by Levan (*Proc. Eighth Int. Cong. Genetics,* 1948) as the basis of a phytoassay in the preliminary screening of active substances. Treatment of onion seedling and bulb root tips for 4 hours and longer in 5 per cent ethyl alcohol approximately 0.005 M mercuric nitrate, 0.1 M and 0.01 M sodium fluoride followed by examination with the Sudan black B technique confirmed essentially results previously reported. The use of the Sudan black B as an adjunct in such assay is considered desirable because of the speed in obtaining the remarkably clear staining of onion chromosomes in which critical prophase stages and other important details are preserved. Comparable results are not usually obtained with a paraffin technique. The large number of nuclei freed from cells throughout the root tip permits an over-all impression of general reaction to the test substance.

Stickiness in various stages of mitosis was produced by the various substances tested. Supercontracted chromosomes produced by 5 per cent ethyl alcohol formed telophase nuclei in which the appearance of the nucleoli were retarded. Mercuric nitrate markedly altered the structure of the interkinetic nuclei producing gross reticula and, in some instances, prevented the new coiling of prophase as shown by heavily nucleinated prophase chromosomes with relic coiling. In the milder responses the chromocenters were accentuated when the interkinetic chromatin did not stain as intensely as the controls.

The use of Sudan black B in the study of heterochromatin in certain plant nuclei. Isadore Cohen.

Following acetic-alcohol fixation a 0.5 per cent solution of Sudan black B in a mixture of formic, propionic, and lactic acids incorporating 50 per cent water stains, brown chromosomes and interkinetic chromatin in temporary smear preparations (Cohen, I., *Stain Tech.* **24**: 177–184, 1949). When observed with a suitable green filter the chromosomes and interkinetic nuclei appear black while the nucleoli do not stain.

Different species of Allium are characterized by the presence of heterochromatic masses, the chromocenters, in their interkinetic nuclei (Levan, A., *Hereditas* **32**: 449–468, 1946). Nuclei of the red onion are relatively free from chromocenters while the nuclei of the bunching onion (Ferry-Morse Seed Company's Evergreen Bunching) show varying numbers of chromocenters. Rapidly dividing nuclei which are heavily nucleinated in the interkinetic stages appear to be free from chromocenters. Nuclei with slower rates of division and nuclei from differentiated cells contain a varying number of chromocenters ranging from 9 to 18. Nuclei containing 14 to 16 chromocenters were more frequently encountered.

The chromocenters stain more intensely than the interkinetic chromatin. In older nuclei the chromocenters reveal an alveolar structure. Based upon the study of prophase and late telophase or early interkinesis, the chromocenters appear to represent in part the uncoiled portions of chromosomes, terminal and centromeric in case of the bunching onion. In the giant nuclei of differential stelar cells, some chromocenters become greatly enlarged and quadripartite at their free ends. Nuclei of onion seedlings killed in 5 per cent ethyl alcohol also show chromocenters with accentuated structure. As a rule, the chromocenters can not be distinguished in prophase after the relic coiling has been abolished and the contraction and new relational coiling have been initiated.

Smears made from the tips of secondary roots of sweet corn (Zea mays) showed the consistent appearance of 3 to 4 large chromocenters and varying numbers of small ones. A pair of larger chromocenters, closely associated with the nucleolus, may well be the nucleolar organizers reported by Morgan (*Jour. Heredity* **34**: 195–198, 1943). The interkinetic nuclei are well-preserved and their chromonematic structure is not obscured as seen in preparations obtained with the paraffin technique.

Root tips of the lima bean (*Phaseolus lunatus*) were similarly examined. The interkinetic nuclei contain on the average 16 chromocenters which are precisely stained with Sudan black B. The chromocenters are connected to very fine chromonemata which are poorly stained, if at all. In prophase, the chromocenters, now visibly double, are incorporated into the long, paired chromatids. Even though the interkinetic and prophase nuclei are somewhat distorted when extruded from the obscuring basophilic cytoplasm, their isolation and the inhibition of nucleolar staining permits a critical examination of details difficult to see by other techniques.

The effect of lithium chloride and calcium low sea water on the development of the otolith of Molgula manhattensis. Arthur L. Colwin.

The tadpole of Molgula normally develops a single sensory organ, an otolith, in the cerebral vesicle. This otolith appears as a black spherical structure.

If eggs in the 4- to 32-cell stages are placed in a solution of lithium chloride 1 : 200 in sea water for 4½ to 5½ hours, the development of the otolith appears to be inhibited and consequently absent in a very large majority of cases. Associated with this treatment is a poor development of the tadpole tail. If treatment with LiCl is not too prolonged a percentage of these tadpoles will metamorphose with the formation of branchial and atrial siphons but the otolith which is normally found between these two siphons is absent.

If eggs are placed in calcium-low sea water within 5 to 10 minutes following fertilization and remain in this solution for from 40 to 90 minutes, the tadpoles which develop may show an increase in the number of pigment spots (otoliths) ranging up to six. Each of these may be of approximately the same size, or smaller or larger than the normal one, or they may be a combination of sizes both smaller and larger than normal. These pigment spots usually appear in a group but not necessarily in the normal location; they may be found in almost any location, even lying superficially in the ectoderm and not in a cerebral vesicle. A small percentage of those treated will metamorphose but the location of the pigment spots may be other than the normal one between the two siphons.

Developmental potencies of the early blastomeres of the egg of Saccoglossus (Dolichoglossus) kowalevskyi. Arthur L. Colwin and Laura Hunter Colwin.

Fertilized eggs within their membranes were treated with calcium-low sea water for varying periods before and during early cleavage. In most cases the early blastomeres separated readily and some remained apart even after being returned to normal sea water.

When the first two blastomeres were separated completely the cleavage pattern of at least the first few cleavages was similar to what it would have been as part of the whole egg. Subsequently two blastulae were formed, giving rise to two gastrulae, and eventually to two complete and perfect larvae: the transverse ciliated band, proboscis, collar, skewing of posterior end, pigmentation, and first pair of gill slits having been formed successively. The time of appearance of these structures corresponds closely with their development in the whole egg. Often complete separation of blastomeres did not occur, or the blastulae derived from separated blastomeres would rejoin. Such cases resulted in various degrees of reduplication, ranging from Siamese twins to two-headed or two-tailed monsters.

When the first four blastomeres were separated completely, the first few cleavages of each quarter appeared as they would have been as part of the whole egg. Subsequently, each formed a blastula and then a gastrula. In the best cases, each proceeded to develop into essentially a miniature of the normal-sized larva. More often the blastomeres or blastulae rejoined, producing one "twin" and two "quadruplets," or one larva of three-fourths size and one "quadruplet," or various two, three or four headed monsters with some parts enlarged or duplicated and other parts missing or reduced in size.

The above evidence suggests that at least each of the first four blastomeres is potentially capable of developing into a normal larva of proportionately smaller size.

The fertilization reaction in the egg of Saccoglossus (Dolichoglossus) kowalevskyi. Laura Hunter Colwin and Arthur L. Colwin.

The mature unfertilized egg of *Saccoglossus kowalevskyi* is about 420 micra long by 330 micra wide, covered by a transparent membrane about 7 micra thick. The densely opaque egg has a narrow border of fine translucent greenish granules or alveoli tightly packed and probably lying within the plasma membrane. The nucleus is in metaphase of the first maturation division.

Upon fertilization the outer membrane moves away from the egg, changing little in appearance. An inner, second membrane becomes apparent and also rises, successively thickening, wrinkling, thinning and smoothing out to a tough spherical structure.

Simultaneously with membrane elevation a wave of agitation sweeps the greenish granules, immediately followed by a wave of "boiling" during which knob-like protuberances arise over the entire periphery of the egg. Each knob consists of an opaque basal mound covered by the greenish granules, now shiny and sending projections outward as if giving off some substance. As "boiling" subsides a fertilization cone forms, similar to, but larger than, one of the knobs. Its clear outer portion presses the second membrane tightly against the first and a thread-like structure appears, extending through both membranes, and connecting cone and sperm head. The thread seems to draw the sperm into the cone. Then the opaque base recedes but a transparent "exudation cone" persists briefly and sometimes even enlarges before it withdraws. The whole process is completed within about 10 minutes of insemination. Meanwhile the greenish granules of the unfertilized egg appear to have given rise to a third extraneous layer consisting of large clear granules and entirely unlike the smooth clear membranes 1 and 2. This third layer is at first separated from the egg by a narrow transparent area crossed by minute striations; later during gastrulation it moves out to lie close to membrane 2, where it persists for some time after the larva starts to rotate.

Cytological investigations of the gut epithelium in haploids and diploids of Habrobracon. D. S. Grosch and A. M. Clark.

Studies of internal tissues indicate that the single-cell layer comprising the wall of the abdominal enlargement of the digestive system should be especially suitable for morphological and cytochemical comparisons between haploids and diploids of Habrobracon. A survey with gut tissues from a two-allele cross 25c × 25 + is being completed. The present report is based on whole mounts fixed with Gilson's fixative, stained by the Feulgen technique (Rafalko modification) and pressed flat when mounted in balsam. An observation important in the light of quantitative nucleic acid studies is that with identical technique the nuclei of "haploid" gut walls are but lightly stained after time intervals of hydrolysis and staining which leave nuclei in "diploids" well colored. This apparently indicates less DNA in the "haploid" nuclei than in the "diploid" nuclei.

Planimeter tracings of camera lucida drawings of 100 cells in each group gave mean nuclear and cell area measurements which show: (1) haploid male nuclei ($25.73 \pm 0.95\,\mu^2$) are smaller than diploid male ($39.92 \pm 1.57\mu^2$) and diploid female ($38.10 \pm 1.38\,\mu^2$) nuclei; (2) haploid male cells ($115.35 \pm 2.76\,\mu^2$) are smaller than diploid male ($173.70 \pm 7.97\,\mu^2$) and diploid female ($155.24 \pm 4.31\,\mu^2$) cells. Comparison of the nuclear per cell ratios among the three groups show them to be statistically similar (0.22 to 0.24).

Previous determinations of cell size based on studies of ommatidia size and on microchaetal counts of wing areas show larger cell areas for diploid males than for females and haploid males, with the latter two groups approximating each other. However, the cell size relationships in the present study indicate that too much reliance should not be placed on any particular tissue.

The time phase of the tide factor hypothesis. Maxwell S. Doty and Justine Garnic.

The earlier published tide factor hypothesis of vertical distribution of intertidal organisms may, for testing purposes, be separated into two components: (1) elevational variations of the critical tide factors, and (2) sudden variations in the time or duration of maximum single emergence or submergence between one level and another. The latter has been investigated by exposing seventeen species of algae to each of three different conditions for periods of time varying from 2 to 96 hours. Often when the time of exposure was doubled or tripled over that time necessary to injure a few thalli, all thalli were killed. One, therefore, is led to accept the hypothesis that it is the tide factors that are responsible for the sharp upward and downward limits of intertidal organisms.

Porphyridium cruentum Nägeli and Porphyridium marinum Kylin. Maxwell S. Doty and Justine Garnic.

During a study of *P. cruentum*, the terrestrial form, some of the material was accidentally dumped into a dish of sea water which upon concentrating by evaporation eventually yielded a rich culture. Following this lead it was found that when grown in 2.5 times the ordinary concentration of sea water with added nitrate and phosphate this reputedly fresh water or terrestrial form grew rather rapidly and with the characteristics attributed by Kylin to his species, including absence of the strongly unilateral sheath. Thus it seems that Kylin's species [1] (a *nomen nudum* per article 45 of the International Rules of Bot. Nomenclature) is not distinct from *P. cruentum* Nägeli.

Pioneer colonization on intertidal transects. Elizabeth M. Fahey and Maxwell S. Doty.

Data from repopulation studies made at Woods Hole, Massachusetts, during the past few years, indicate that irrespective of the time of clearing or exposure a certain set of rapidly-growing organisms, called pioneer organisms, are the first macroscopic forms to appear. Transects cleared in the summer or fall have become populated with pioneer forms of Enteromorpha, Polysiphonia and occasionally other genera. These forms, while relatively short-lived or transient reproduce actively throughout these seasons. They are almost constantly present in the neighborhood but on the transects they may reach maturity and disappear in two months time. Late winter clearings, on the other hand, have been repopulated first by long-lived forms, e.g., Balanus, the reproductory periods of which are restricted largely to this part of the year. On these transects such forms as Enteromorpha failed to appear.

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[1] Kungl., *Fysiografiska Sällskapets Lund Förhandlingar* 7 (10): 1–5, 1937.

Slowly growing organisms, e.g., Scytosiphon, Chordaria, Nemalion, etc., tend to be the next occupants of cleared transects. They may succeed the transient pioneers on the rocks or in the case of the presence of the long-lived forms they may be epiphytic or epizotic forms, e.g., Ralfsia occurring on Balanus or the various gastropods feeding on the algae. Slowly maturing, long-lived organisms such as Fucus in time come to dominate. Since such communities tend to perpetuate themselves they may be considered as analogous to the "climax" forms of terrestrial ecologists.

The current observations tend to support the hypothesis that the course of repopulation insofar as it concerns any particular succession of species is dependent on the life cycles and forms of the organisms present as well as the time of clearing in respect to the time of reproduction particularly of the rapidly-growing longer-lived organisms. Thus, a classification of the species involved should include a consideration of at least three characteristics of the species; namely: (1) growth rate; (2) time of reproduction; (3) life cycle and life forms.

The aforementioned classification would probably be best treated in terms of a succession involving: (1) Pioneer colonization by rapidly-growing forms which may be transient as Enteromorpha or persistent as Balanus; (2) colonization by more slowly-growing, seasonal, usually non-climax forms; and (3) climax colonization by long-lived or slowly-growing forms which reproduce or reestablish themselves, e.g., Fucus and Balanus.

Intervarietal mating reactions in Paramecium caudatum. LAUREN C. GILMAN.

In collections of *Paramecium caudatum* from various localities chiefly in the states of Maryland, Pennsylvania, Massachusetts, South Dakota, and Connecticut ten varieties numbered 1 to 10 and consisting of mating types I to XVII and XX were found. So far no type XIX to go with type XX has been discovered. Each variety consisted of two mating types which gave, when mixed under the appropriate conditions, close to 100 per cent immediate agglutinative mating reaction followed by a proportionate amount of conjugation. In general conjugation occurred only within a variety although a number of exceptions were found.

In making the tests between the varieties controls consisting of unmixed samples were set up as were also controls consisting of mixtures of the two mating types in each variety. In order for the results of the mixtures to be significant it was necessary that no conjugation occur in the unmixed controls and that the control mixtures give close to 100 per cent mating reaction followed by conjugation in proportionate amounts. The amount of conjugation was estimated roughly by visual inspection of the mixtures.

No cross reactions were found with varieties 1, 4, 5, and 7. Variety 3 gave a cross reaction with variety 6. Cross reactions also occurred between varieties 2, 8, 9, and 10. The cross reactions encountered were of two kinds. The first kind consisted of a weak mating reaction involving about 5 per cent of the animals and resulting in no conjugation. The second kind consisted of mating reactions as strong as those found within a variety which were followed by proportionate amounts of conjugation. The first kind of reactions was given by the following: type VI variety 3 with type XI variety 6; type III variety 2 with type XVI variety 8; type XVIII variety 9 and type XX variety 10; type XVII variety 9 with type IV variety 2 and type XVI variety 8.

Intervarietal mating reactions in *Paramecium caudatum* reaction was given by the following: type XV variety 8 with type IV variety 2 and type XX variety 10; type XVII variety 9 with type XX variety 10. If the animals were not in optimal condition giving close to 100 per cent mating reaction at the time of mixture the first kind of reaction was completely eliminated and the second kind proportionately reduced.

The effect of temperature on growth and sexual changes in Crepidula plana. HARLEY N. GOULD.

Studies made at Woods Hole during the summer and fall of 1948 showed changes in the rate of growth and sex transformations in *Crepidula plana*. Growth of young, development of the male phase, regression of the male phase following removal from the vicinity of females, subsequent growth and onset of the female phase are all accelerated up to the end of August. The rate of these changes falls off rather suddenly in September and progressively thereafter, communities of the limpets reaching an almost static condition by the first of December. Many factors of the environment are changing, one of which is the temperature of the sea water. The temperature of the surface water in the harbor reached 22 degrees C. in August, 1948, and decreased to 10 degrees by the first of December.

To determine the effect of temperature as distinguished from other environmental factors, parallel sets of cultures, of similar size and sexual condition were kept during July, 1949, in running sea water at different temperatures: control at 22 to 23 degrees and experimental at 12 to 16 degrees.

Small sexless young in absence of females show rapid growth at normal temperature, very retarded growth at reduced temperature. Spontaneous male development is partial at normal temperature, completely absent at reduced temperature.

Similar small sexless young confined with mature females show retarded growth at normal temperature, almost none at reduced temperature. Induced male development under these conditions is complete in most specimens at normal temperature, incomplete or absent at reduced temperature.

Males removed from the vicinity of females lose their male character quickly and grow rapidly at normal temperature, but retain male organs longer and grow slowly at reduced temperature.

It is concluded that temperature is a major factor influencing growth and sexual changes even when the summer food supply in the sea water is available.

Oxygen utilization in relation to growth and differentiation in the slime mold Dictyostelium discoideum. James H. Gregg.

The growth and morphogenetic stages of the slime mold *Dictyostelium discoideum* are separate during the life cycle. The rates of oxygen consumption of the independent amoebae of the growth stage and the migrating, preculmination, and culmination stages of morphogenesis were determined. The oxygen consumption measurements were made with a microrespirometer. The independent amoebae and the individual slime molds of the morphogentic stages were analyzed for total nitrogen by a microKjeldahl method in order that the oxygen consumption measurements could be expressed in terms of a unit mass of nitrogen.

It was found that the morphogenetic stages respired at a greater rate than the growth stages. The individual slime molds of the morphogenetic stages vary greatly in size. Bonner and Eldredge (*Growth,* Vol. IX, No. 4, 1945) found that the larger slime molds culminated at a greater rate than the smaller slime molds. The oxygen consumption of the various sized slime molds was identical, within the experimental error, per unit mass of nitrogen. Therefore, the smaller slime molds require more oxygen per mm. of culmination per unit mass of nitrogen than the larger slime molds. The rate of respiration during the transition of the slime mold from the preculmination stage to the completely culminated stage was determined. The rate of respiration remained constant until the latter part of culmination when a slight decline in respiration began which continued until the slime mold ceased completely to respire. An examination of the culmination rate has shown that the culmination declines simultaneously as the respiration decreases.

It is suggested that the morphogenetic processes of the slime molds require energy above that required for growth and maintenance. In view of the parallel between the rates of culmination and respiration it is suggested that respiration and morphogenesis are coupled in this organism. This is further indicated by the fact that when morphogenesis ceases respiration also ceases.

Vitamin K as a protoplasmic coagulant and parthenogenetic agent. Atida Halaban.

If protoplasmic clotting is similar to blood clotting and if protoplasmic clotting or gelation is a preliminary to the appearance of the mitotic spindle and cell division, then it might be reasoned that substances with vitamin K activity which play a role in blood coagulation might have an effect on the protoplasm of the cell and the initiation of mitosis.

The experiments were done on *Arbacia punctulata* eggs. Dilute solutions of 2-methyl-1,4-naphthoquinone were found to cause a high percentage of artificial parthenogenesis and they also produce a gelation of the egg protoplasm. Arbacia eggs were exposed to solutions of the following concentrations: 10 mg. per liter, 5 mg. per liter and 3 mg. per liter of 2-methyl-1,4-naphthoquinone in sea water for 5 to 60 minutes; they were then washed and removed to sea water at 5 minute intervals. Counts of dividing cells were made after 4 hours. An exposure of 15 minutes to the 10 mg. per liter solution gave 70 per cent parthenogenesis. Exposure to this concentration for 30 minutes results in sharp protoplasmic gelation. A 30 minute exposure to a concentration of 5 mg. per liter causes 90 per cent of the eggs to cleave. Lower concentrations require longer exposure times for a maximum percentage of cleavage.

When eggs were exposed to a concentration of 10 mg. per liter, an increase in viscosity occurred following 15 to 25 minutes of exposure and gelation was complete after 30 minutes. If after 20 minutes in the solution the eggs were transferred to sea water, there was a progressive increase in protoplasmic viscosity, so that by the end of 2 hours the protoplasm was completely gelled. Cell division followed this gelation.

Effect of ultraviolet light (2537 Å) *on cleavage time in centrifuged Arbacia eggs.* CLIFFORD V. HARDING[1] AND LYELL J. THOMAS, JR.

Arbacia eggs were centrifuged for one minute in an Emerson hand centrifuge (9000 times gravity). This treatment is sufficient to cause distinct stratification of the visible components. A small amount of isotonic sucrose was put in the centrifuge tubes to prevent crushing of the eggs against the bottom. These eggs were then placed upon a layer of isotonic sucrose (4 mm. in depth) in each of two quartz petri dishes. After two minutes the majority of the eggs became oriented with the fat cap at the top and the pigment layer at the bottom. One petri dish was then placed above the ultraviolet lamp and the other below the lamp (Westinghouse Sterilamp, 2537 Å; dose, 720 ergs per mm.^{2}). The two dishes were irradiated simultaneously. The eggs were then washed three times in sea water and fertilized about 15 minutes after the exposure. All the eggs were kept under the same conditions of temperature (approximately 23° C.) and light until counted for first cleavages. In every experiment the eggs irradiated through the fat cap (those eggs placed below the lamp) cleaved after those irradiated through the pigment layer. In experiments carried out under the conditions referred to above this difference in cleavage time averaged 11.5 minutes.

The distances of the petri dishes from the source of ultraviolet light were so adjusted that uncentrifuged eggs showed no significant differences in cleavage time between those irradiated from above and those irradiated from below. It seems, therefore, that the centrifuged eggs are truly more sensitive to the ultraviolet light when irradiated through the fat cap.

Initiation of cell division by injury substances.[2] DRUSILLA HARDING.

Extracts of injured tissues from the frog and clam were tested for their effectiveness in causing artificial parthenogenesis in the Arbacia egg. Injury substances were prepared according to the method described by Heilbrunn et al. in their studies of heat death (*Physiol. Zool.* **19**: 404–429, 1946). The freezing point lowering of the solution was adjusted to slightly less than that of sea water to prevent activation by hypertonicity. The pH of the solution was between 4 and 5. In every case, the extracts caused parthenogenesis. Neutralization of the extracts, however, caused loss of parthenogenetic activity. The effectiveness of these injury substances was compared with the effectiveness of various acids at the same pH, and the following results obtained: The averages of the highest per cent cleavage from each experiment were for the extract, 27.08, for the butyric acid, 21.18, for acetic acid, 22.95, for lactic acid, 3.05, and for phosphoric acid, 1.7. The per cent cleavage in the extracts is significantly higher than in the other acids. The percent cleavage in butyric acid is not significantly different from that in acetic acid.

Viscosity determinations showed that after an exposure of the eggs to the extract just long enough to produce the first signs of division, an increase in viscosity can be detected. The viscosity increases about 15 minutes after removal of the eggs to sea water, and rises as much as 4-fold by 50 minutes.

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[1] Public Health Service Research Fellow of the National Institutes of Health, Department of Zoology, University of Pennsylvania.

[2] Aided by a grant from the U. S. Public Health Service administered by L. V. Heilbrunn.

It is concluded from these results that an acid substance is released as the result of injury. This acid of injury can cause an increase in viscosity and initiate cell division in the Arbacia egg.

Cell division in relation to protoplasmic clotting.[1] L. V. Heilbrunn and W. L. Wilson.

When a cell is incited to divide, the primary change is believed to be a protoplasmic clotting or gelation, and this clotting or gelation is thought to be essentially similar to the clotting which occurs in blood. Earlier work on the egg of the worm Chaetopterus has shown that heparin prevents mitotic gelation and also prevents division of the cell. Similar results have now been obtained with preparations of the bacterial polysaccharide which Shear found so effective in causing regression of tumors in mice and rats. When egg cells are placed in solutions of the polysaccharide 2 minutes after fertilization, mitosis is inhibited and the protoplasm instead of undergoing normal mitotic gelation remains fluid.

Other agents which influence blood clotting have a marked effect on the division of the Chaetopterus egg cell. Thus, a vitamin K, 2-methyl-1,4-naphthoquinone, in very high dilution can suppress cell division. Concentrations of 1 mg. per liter are sufficient to produce this effect. In such concentrations, the protoplasm remains fluid for hours but eventually becomes very much more viscous than normal. In higher concentrations of vitamin K, the mitotic gelation is suppressed for a time, but then the protoplasmic viscosity rises to very high levels. The eggs do not recover after such treatment.

Dicumarol also has a marked effect on cell division. A solution containing 100 mg. per liter completely stops cell division. In such a solution, the mitotic gelation is maintained and does not reverse at the normal time. The protoplasm of the cell remains more viscous for an hour or two and then the protoplasm becomes fluid and remains fluid. Typically, after a long delay the eggs in dicumarol solution undergo a single mitotic division and then stop. The effect is reversible and eggs removed from the dicumarol solution proceed to divide and develop.

Oxidative phosphorylation by a cell-free particulate enzyme system from unfertilized Arbacia eggs. A. K. Keltch, C. F. Strittmatter, C. P. Walters and G. H. A. Clowes.

Preparation of the particulate enzyme system was carried out as follows: Unfertilized Arbacia eggs were washed once with 100 volumes sea water, three times with 100 volumes of a solution 0.5 M in NaCl, 0.02 M in $NaHCO_3$, pH 7.9, and collected by centrifuging 30 seconds at 1000 g. in chilled cups; all subsequent operations prior to incubation were made at 5° C. The packed eggs were mixed with 5 volumes of a solution 0.4 M in KCl, 0.01 M in sodium citrate, 0.05 M in glycylglycine, pH 7.4; this suspension was forced three times through a No. 18 needle against a glass surface, 95 per cent or more of the eggs being broken up. The resulting suspension of egg fragments was spun 8 minutes at 1000 g. in a refrigerated centrifuge (angle head). The top, pinkish-white layer was used as the particulate enzyme system effecting oxidative phosphorylation.

The concentrations of reagents in each Warburg flask were (in mM per liter): KCl, 57; citrate, 1.4; $MgCl_2$, 14; NaF, 36; inorganic phosphate, 1.4; glycylglycine, 27; glucose, 36; substrate, 10; nicotinamide, 3.6; diphosphopyridine nucleotide, 0.2; adenosine triphosphate, 0.7; cytochrome C, 0.014. The total volume was 2.8 ml., including 0.2 ml. yeast hexokinase solution and 0.6 ml. egg particulate enzyme, which was added last.

The flasks were incubated, with shaking, for 1 hour at 20° C. One ml. aliquots were fixed with 2.6 ml. cold, 11 per cent trichloroacetic acid; the inorganic phosphate was determined according to Fiske and SubbaRow. Phosphorus utilization was ascertained by comparison with appropriate controls fixed before incubation. The average initial inorganic phosphorus content of each flask was 175 micrograms, of which 96 were added. The source of the rest is undefined.

This crude particulate enzyme system can carry out oxidative phosphorylation with various substances of the tricarboxylic acid cycle as substrate. In a typical example, oxygen consumption (c.mm.) and phosphate uptake (micrograms) per flask per hour were: no substrate: O_2, 24, P uptake, 13; α-ketoglutarate: O_2, 34, P uptake, 101; oxalacetate: O_2, 30, P uptake, 91; succinate: O_2, 31, P uptake, 53.

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[1] Aided by a grant from the U. S. Public Health Service.

These data indicate that the Arbacia egg can generate high-energy phosphate bonds by use of substrates of the tricarboxylic acid cycle.

The authors thank Dr. M. E. Krahl for advice.

Electrographic observations on seagulls. BRUNO KISCH.

Eight seagulls have been investigated and standard leads, chest leads, and in five cases direct leads from the surface of the heart have been taken.

The heart rate of the narcotized animals (ether) is between 450 and 520 beats per minute as long as the heart beat is normal.

In each of the investigated cases the voltage of lead I is very small and lead II and III very similar, both showing a deep Q-S or a very small R and a deep S, resulting in a very definite left axis deviation. The right chest lead equals very much the left chest lead. The entire picture is very similar to the conditions previously described for the calf.

By taking direct leads from the heart surface using for an indifferent electrode, a Wilson central terminal, the cardiogram was found as follows: the right auricle shows a small R, deep S type; the left ventricle a high R, no S type. From the surface of the right and left ventricle a Q-S, or small R, deep S can be registered from the pectoral surface, but from the dorsal surface a deep Q-S.

Using the blotting paper method, auricular fibrillation and auricular flutter could be produced by application of acetylcholine to the right auricle.

The haematologic findings in the seagulls were as follows: Red blood count average 2.7 ml., maximum 3.1, minimum 2.2, haematocrit reading 34 per cent, haemoglobin content 10.4 g. per cent, size of red blood cells average 14.1 × 8.1 μ, maximum 18.3 × 8.6, minimum 10.8 × 8.1.

A detailed report with tracings will be published in "Experimental Medicine and Surgery."

An unknown type of haemolysis. BRUNO KISCH.

Among thirteen eels haemotologically investigated were three sick or dying brought in by fishermen. Only in the blood of these three animals were found, besides the normal red blood cells, red blood cells containing one or more crystal-like inclusions in the form of long prismatic or needle-like bodies. These inclusions show microscopically the same color and similar properties as the content of the normal red blood cell. In growing they stretch the membrane of the red blood cell, which reached up to twice the diameter of a normal cell (for instance: 28 μ instead of 14 μ). The membrane finally ruptures. It remains a long prismatic shaped body with the attached nucleus, which retains its normal form and size. Never were these bodies found without nuclei, so probably, after the rupture of the membrane they may be dissolved, and the isolated nucleus remains.

The here described crystal-like shaped formations are probably not haemoglobin crystals because their color is not darker than the contents of the erythrocytes.

An extensive report will be published.

Genetics of Chlamydomonas—paving the way. RALPH A. LEWIN.

Problems of photosynthesis may be attacked by the methods of biochemical genetics using a chlorophyllose micro-organism which can be grown rapidly in pure culture, and in which sexual reproduction can be controlled.

Complementary mating types of *Chlamydomonas Moewusii* Gerloff have been isolated by L. Provasoli, and the life cycle can now be experimentally reproduced. The alga is routinely grown on 1 per cent agar to which mineral salts (Beijerinck's) and 0.1 per cent sodium acetate are added. Suspensions of sexually active cells can be obtained by flooding 2 to 5 day old cultures with water. The processes of clumping and pairing require light, and a minimum of 14 hours (at 470 f.c.) has been found necessary for complete fusion of gamete protoplasts. Only a very small percentage of zygotes which have been matured under continuous illumination can be made to germinate, even after a dormancy period of 1 to 2 months: many chemical and physical treatments have been tried to "break" dormancy, without success. On the other hand, zygotes placed in darkness 14 to 28 hours after mating germinate regularly within 6 to 10 days. Zoospores are liberated on flooding, and may give rise to isolated clonal colonies. Tetrad analysis is carried out as described by Moewus (*Zeit. Ind. Abst. Verh.* **78**: 418–522, 1940).

Mutations have been obtained by ultra-violet irradiation using standard screening techniques. In addition to numerous slow-growing and palmelloid clones, the following mutants have been isolated: M.67 "Flagella-less." Sexually sterile. M.151 "Non-photosynthetic." Pigments apparently normal, but growth negligible in absence of organic carbon source. M.236 "Paralysed." Most cells unable to swim, though some may swim abnormally with slow flagellar beat. Typically flagella remain extended and rigid except for twitching of tip. Cells may progress over glass slide (2 mμ per second), drawn by peculiar serpentine movement of flagellar tip in contact with substrate. No artificial stimulation of flagellar movement achieved to date.

Genetic studies using these mutants are now in progress.

Recovery from ultra-violet light induced delay in cleavage of Arbacia eggs by irradiation with visible light. ALFRED MARSHAK.

Irradiation of Arbacia sperm with ultra-violet light 2537 Å, 52 microwatts/cm. for 30 seconds approximately doubled time for cleavage of eggs fertilized with this sperm. Increasing the ultra-violet dose increased the delay. Six times this dose given to eggs produced only 20 per cent delay, which suggests the effect is on the nucleus. Following ultra-violet irradiation with intense visible light (28,000 ft. candles) of either sperm or unfertilized eggs had no effect, neither did visible light alone affect the gametes. Visible light given to the zygote markedly reduced the delay in cleavage time. The effect was observed when the light was given 2 to 50 minutes after fertilization, but maximum efficiency was at 9 to 11 minutes. Significant reactivation was obtained with light 560 mμ–620 mμ (max. 570 mμ) (1.6 per cent total transmission), but light 330 mμ–470 mμ (max. 440 mμ) (total transmission 0.1 per cent) was more effective. Increasing exposure time gave increasing effect but a maximum was reached at 3 minutes. Following ultra-violet treatment of sperm, the delay-producing factors increased with time after irradiation. In the unfertilized egg, there was neither growth nor decay of the delaying factors. Photoreactivation was obtained with fertilized nucleated half-eggs containing no pigment granules as well as with whole eggs.

Because of their probable connection with nuclear metabolism, eggs, sperm, and zygotes were treated with the following substances before, during, and after irradiation with ultra-violet and visible light: adenosine, streptomycin, sodium usnate, folic acid, 4-amino n-methyl folic acid, and 2,4-diamino 5-p-chlorophenoxypyrimidine. None had any effect on either ultra-violet inactivation or photoreactivation. Riboflavin also had no effect on reactivation of the zygote. Sperm irradiated with visible light soon became immobile. Eggs could be fertilized before immobilization and although the sperm aster formed in the usual time, cleavage was much delayed and abnormal, but this effect seems unrelated to the photoreactivation phenomenon.

No delay in polar body formation or in cleavage was found in Chaetopterus eggs fertilized with sperm irradiated with 6 times the ultra-violet dose given Arbacia.

The effect of necrosin on the cleavage for fertilized sea urchin ova. VALY MENKIN AND LOUISE A. PIROVANE.[1]

The writer has demonstrated that injured cells of higher animals liberate in inflammatory exudates a toxic substance which per se offers a reasonable explanation for the pattern of injury in inflammation. This substance is either a euglobulin, or else it is associated with the euglobulin of particularly acid exudates. It has been termed necrosin (*Arch. Path.* **36**: 269, 1943; **39**: 28, 1945). Subsequently it was shown that this toxic substance, or one closely resembling it, is also liberated in the severely injured cells of invertebrates such as *M. arenaria* or *L. polyphemus* (*Physiol. Zoology,* **32**: 124, 1949).

These studies have been continued to determine whether necrosin has any effect on cell division of the unfertilized and fertilized ova of *Arbacia punctulata.* Necrosin has often a reduced activity when in the lyophilized state. This, however, is not always the case. The

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[1] Aided by a grant from the National Advisory Cancer Council.

studies were carried on with an active dry frozen preparation, previously assayed in the cutaneous tissue of a rabbit. About 10 milligrams of necrosin were added to a standard dish containing 10 cc. of sea water and 2 cc. of suspension of Arbacia ova. The ova tended soon to agglutinate, cytolyze, or fuse. They often assumed a deep red color. In other standard dishes, 5 drops of Arbacia sperm were added, and the time and percentage of segmental division observed. It was noted in a large number of experiments that the division of the fertilized eggs was definitely retarded upon the addition of necrosin. However, once division had been initiated, the addition of necrosin failed to retard the process. The retarded division was at times, but not always, accompanied by abnormal cleavage. Necrosin was also quite toxic to the spermatozoa, so that the percentage of fertilized ova was diminished. The necrosin was originally obtained from dogs. The substitution of another canine globulin, namely the leukocytosis-promoting factor (LPF), failed to inhibit the rate of cleavage.

Mating reactions between living and lyophilized paramecia of opposite mating type.[1]
Charles B. Metz and Edna M. Fusco.

Since dead paramecia can give specific mating reactions with living animals of opposite mating type (Metz, 1946), it seemed likely that paramecia could be dried without complete destruction of mating reactivity. This proved to be the case with both *P. aurelia* and *P. calkinsi.* Reactive *P. aurelia* were killed with formalin, washed in saline, quickly frozen in a solid CO_2-acetone bath and finally dried from the frozen state (lyophilized). When suspended in saline the dried *P. aurelia* clumped strongly and specifically with living animals of opposite type. Only weak reactions were obtained with *P. aurelia* that were frozen in the living condition. *P. calkinsi* must be frozen while alive and then lyophilized. Formalin killed lyophilized *P. calkinsi* give only weak reactions. Properly dried *P. calkinsi* (but not *P. aurelia*) retain their mating reactivity for several months if not indefinitely at room temperature.

Since lyophilized *P. aurelia* not only give mating reactions but also induce macronuclear breakdown and pseudo selfing pair formation in living animals of opposite type, the activation initiating mechanism (Metz and Foley, in press) is not destroyed by lyophilization. This is in agreement with the view that interaction of mating type substances initiates activation.

Technically the procedure makes possible: (1) study of the effect of anhydrous solvents on the mating substances, (2) electron microscope study of paramecia that are known to have reactive surfaces and (3) storage of reactive animals. So far only the first of these possibilities has been explored. Thus the mating reactivity of dried paramecia (aurelia or calkinsi) is not destroyed by extraction (30 minutes, room temperature) with absolute ether, acetone, benzene or chloroform. Absolute alcohol inactivates the paramecia. These results would seem to eliminate loosely-bound fat-soluble substances as essential constituents of the mating type substance(s).

The protoplasmic viscosity of muscle and nerve.[2] Peter Rieser.

Small oil drops were microinjected into frog muscle fibers, and the rise of the drops through the protoplasm was observed in a horizontal microscope. The protoplasmic viscosity was determined from Stokes' law. An average value of 29 centipoises was obtained. Some higher values were interpreted as representing cases where a slight degree of injury had occurred. Oil drops were able to move only in one direction through the fibers. No movement across the fibers was ever observed. These facts, as well as the ovoid shape of the drops, suggest the existence of some longitudinal organization within the fibers. Microinjection of large masses of oil or air always showed that a peripheral region directly within the sarcolemma could never be displaced by these substances. By microinjection of aqueous solutions it was possible to push the protoplasm ahead through the fibers. In every instance where such a flow occurred there was a peripheral region of approximately the same thickness as obtained by the oil and air displacement method, and this peripheral region did not flow. This outer region had a minimal thickness of approximately 10 μ. The outer region thus appears to be similar to the gel-like cortex of other types of cells. Preliminary studies on oil drops injected into fibers from the ventral chain of the lobster reveal the fluid nature of the axoplasm. The average viscosity was 5.5 centipoises. The shape of these drops, unlike those injected into squid axoplasm, was spherical. The oil drops were not able to move in the opposite direction, and no movement across the fibers was ever noted. In the giant axon of the squid no movement of oil drops could be observed; this is perhaps due to the greater sensitivity of squid axoplasm.

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[1] Aided by a grant from the National Institute of Health, U. S. Public Health Service.

[2] Aided by a grant from the U. S. Public Health Service administered by L. V. Heilbrunn.

The use of extra-polar stimulus escape to measure nerve membrane characteristics. OTTO H. SCHMITT.

It is possible to show on simple theoretical grounds that a sinusoidal stimulus applied to a nerve between two ringlet electrodes should produce a signal in the extrapolar regions which decays exactly exponentially and which changes in phase linearly with distance. It is further possible to show that these two attenuation constants contain all the data needed beside longitudinal nerve resistance to determine quantitatively at each test frequency the membrane conductance, membrane susceptance, and phase velocity of propagation along the nerve. These predictions are extremely well borne out by experiment and besides verification of certain previous measurements, considerable new information regarding the effects of drugs and ions is emerging. For squid nerve a membrane capacitance of 1 to 1.5 mfd/cm.^{2} is uniformly found at 500 to 1000 c.p.s. Conductance for normal nerve in the 200 c.p.s. region is about 1 millimho/cm.^{2} but in the mid-frequency region around 500 c.p.s. total conductance of the membrane drops to near zero and seems to dip into the negative region under the influence of calcium. A strong reactive component in the 75 to 300 c.p.s. region is found which is quite sensitive to drugs and which can be pushed far into the positive susceptance region, especially by calcium and veratrine. It is also possible to demonstrate progressive changes in the membrane and response to drugs long after the nerve has ceased to respond and after conductivity of the membrane has increased many fold.

Because the method is not limited to single fiber preparations and does not require giant axons, it is foreseen as a valuable means for determining electrical characteristics of nerve preparations unsuitable for single fiber study and its extension to muscle experimentation seems feasible.

Glucose metabolism in marine Annelids. ELIZABETH SETON AND CHARLES G. WILBER.

Previously published evidence indicates that an increase in the environmental temperature of the Sipunculid, *Phascolosoma gouldii,* is accompanied by an increase in the amount of glucose in the coelomic fluid. Further work was done in an attempt to ascertain whether a similar pattern obtains in other marine worms. *Amphitrite ornata* was exposed to various temperatures for different periods of time and the coelomic fluid then removed and analyzed for glucose. In specimens exposed to 32° for times up to 12 hours, there is an appreciable increase in coelomic fluid glucose. If time in hours is plotted against concentration of glucose for a given temperature, a curve is obtained which rises from 32 mg. per 100 cc. (control value) and approaches asymptotically a maximum of 66 mg. per 100 cc. A similar curve plotted for Phascolosoma rises from the control value of 17 mg. per 100 cc. and approaches a maximum of 36 mg. per 100 cc. The calculated temperature coefficient for the process in Amphitrite varies between 1.3 and 2.0. Exposures to 38° results in a tremendous increase of fluid glucose in Amphitrite, and in first a marked decrease and then an increase of glucose in Phascolosoma. The origin of the glucose is not known at this time.

Effect of arsonoacetic, trans 1,2, cyclopentanedicarboxylic, and β-phosphonopropionic acids on enzyme systems in the ciliate, Colpidium campylum. GERALD R. SEAMAN AND ROBERT K. HOULIHAN.

Klotz and Tietze recently (*J. B. C.* **168**: 399) synthesized phosphonate, arsonate, and sulfonate analogues of succinic and malonic acids as well as cyclic analogues. Their results obtained with rat liver indicate that only the sulfonate analogues are capable of interacting strongly with succinic dehydrogenase. It therefore seemed desirable to test the efficiency of some of these analogues using the ciliate protozoan, *Colpidium campylum*. The membrane of Colpidium is impermeable to succinate. Tissue homogenates were therefore used.

At inhibitor-substrate ratios of 1 : 2, β-phosphonopropionic, malonic, *trans* 1,2, cyclopentanedicarboxylic, and arsonoacetic acids cause inhibitions of 15, 19, 41, and 47 per cent, respectively, of the oxidation of succinate (0.02 M). At ratios of 2 : 1 the inhibitions are 58, 59, 85, and 98 per cent respectively. The increased degree of inhibition found when the inhibitor-substrate ratio is increased indicates that the inhibitions are competitive.

To obtain indications as to the specificity of the action of the compounds, pyruvate and acetate were used as substrates (in concentrations of 0.02 M), using living cells rather than homogenates. In inhibitor-substrate ratios of 2 : 1, malonate, phosphonate, and arsonate cause inhibition of acetate oxidation by 38, 57, and 100 per cent respectively. When pyruvate is the substrate, the inhibitions 51, 63, and 83 per cent, respectively. Therefore, the inhibitions are probably specific in their locus of action. Cyclopentanedicarboxylic acid, rather than inhibiting, accelerates the oxidation of pyruvate and acetate by 41 and 39 per cent respectively.

When living cells are incubated with succinate, succinate plus arsonate, malonate, or phosphonate, there is no increase in oxygen uptake due to the impermeability of the cell membrane to succinate. However, with cyclopentane and succinate, there is an increased oxygen uptake of 47 per cent. This seems to indicate that *trans* 1,2, cyclopentanedicarboxylic acid, in some manner, increases the permeability of the cell membrane of *Colpidium* and thus allows succinate to enter.

Electrical changes in crab nerve in relation to potassium movement.[1] ABRAHAM M. SHANES.[2]

The depolarizations of the leg nerves of *Libinia emarginata* during anoxia and during repetitive stimulation, as well as the repolarizations during recovery, are found to have certain similarities indicative of a common underlying process. Thus, the potential changes are augmented by veratrine and altered by differences in the volume of solution in contact with the fibers. These and other considerations (Shanes and Hopkins, 1948) suggest that the basis for the polarization fluctuations is alteration in the mechanism of potassium retention. In keeping with this the potassium lost per impulse computed from the potential changes with stimulation is of the same order as the available analytical figures.

For anoxia, however, the literature (Cowan, 1934) indicates no potassium loss. This has therefore been reexamined by flame spectrophotometric analyses[3] of ca. 1 cc. samples of sea water successively put in contact with the same set of 3 or 4 nerves for half hour intervals. Typically, 2 samples were taken first in oxygen, then 2 in nitrogen, and finally another 2 in oxygen again. In all cases a loss of ca. 20 μM per gm. wet weight per hr. occurred in oxygen whether or not glucose was present; anoxia doubled or trebled this leakage and 50 to 100 mm. glucose reduced the increment; return to oxygen either completely stopped the potassium escape or actually caused an absorption of potassium, the recovery being less striking when glucose was present.

These analytical data are in complete accord with electrical measurements carried on under the same experimental conditions. Thus, the marked depolarization during anoxia is appreciably reduced by glucose and the amplitude of the repolarization in oxygen in correspondingly less.

Replacement of 50 to 75 per cent of the sodium of an artificial sea water with choline markedly and reversibly reduces the degree of depolarization occurring with anoxia or stimulation; an equivalent amount of choline chloride has no such effect when the sodium content of the medium is unaltered. These and other experiments suggest that the potassium escape occurs by exchange with extracellular sodium, possibly through failure of a sodium exclusion mechanism. Further study is needed, however, and is presently in progress.

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[2] Department of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D. C.

[3] Dr. George Marmont very generously permitted the use of a Beckman flame spectrophotometer.

The chloride content of frog muscle. W. D. Shenk.

There has been growing evidence that the Van Slyke method of chloride determination, a method which involves wet washing, is inadequate for muscle. Accordingly, an attempt has been made to determine the merits of the Parr bomb method for chloride determinations in whole muscle. The principle of the method consists in burning the muscle with sodium peroxide in a Parr bomb. In order to accomplish the burning of wet muscle, the charge, consisting of sodium peroxide, potassium nitrate and benzoic acid is first carefully mixed on a watch crystal. The ignition cup is then layered with this mixture, the muscle carefully centered in the cup and the remaining charge added and ignited. After ignition, the solid mass of residue is dissolved in warm distilled water and is rendered neutral with concentrated nitric acid; 5 ml. of excess acid is then added. After the addition of a controlled amount of silver nitrate the solution is boiled for one hour to coagulate the silver chloride, cooled to room temperature, filtered, washed twice, and the washings added to the filtrate. The filtrate is then titrated with 0.01 N ammonium thiocyanate until one drop causes a color change which persists for one minute at room temperature.

A marked difference was noted in comparing the values obtained by analyzing small pieces of the same muscle using the Parr bomb method in contrast with the Van Slyke technique. Analysis of wet muscle after acid digestion gave an average of 21.016 milliequivalents of chloride per kilogram, whereas the Parr bomb determinations gave an average of 39.4 milliequivalents per kilogram. Dry muscle gave 57.2 milliequivalents of chloride per kilogram with the Van Slyke method and 130.35 milliequivalents with the Parr bomb method.

It is believed that muscle must contain bound chloride which resists detection by customary analytical procedures.

The photosensitive pigment of the squid retina. Robert C. C. St. George and George Wald.[1]

The retina of the squid, *Loligo pealii,* contains a high concentration of retinene$_1$, a precursor for the synthesis of rhodopsin and a product of its bleaching in the vertebrate eye. In the squid retina, retinene$_1$ is found both free and bound to protein; and illumination of the dark adapted retina releases retinene$_1$. From these data it was concluded that the squid retina contains a photosensitive pigment closely related to rhodopsin, and like it capable of bleaching in the light to yield retinene$_1$ (Wald, *Amer. J. Physiol.* **133**: 479, 1941; *Biol. Symp.,* VII, 43, 1942).

Recently Bliss has extracted a red pigment ("cephalopsin") from the squid retina which resembles rhodopsin in its visible absorption spectrum, and which yields retinene$_1$ on chemical destruction. Bliss suggests that this may be the visual pigment of the squid, but states that though it decomposes rapidly in the dark, it is not affected by light (*J. Biol. Chem.* **176**: 563, 1948). It could not, therefore, be the source of the retinene$_1$ liberated by light from the retina; nor is it clear how a pigment that is not affected by light can be responsible for vision.

We have now examined the behavior of a pigment extracted from squid retinal rods by a procedure not materially different from that used by Bliss, and used by us earlier in the preparation of frog and cattle rhodopsin. Its absorption spectrum, like that of rhodopsin, consists of three bands: an α-band at 490 mμ, a low β-band at 365 mμ, and a high protein γ-band at 279 mμ. It is stable for many hours at room temperature in darkness. In the light it undergoes a photochemical change followed by a "dark" reaction, comparable with the transformation of rhodopsin to lumi- and to meta-rhodopsin (Wald, Durell and St. George, *Science,* in press). At temperatures above 23° C. these processes initiated by light are followed by bleaching in darkness to a mixture of retinene$_1$ and the regenerated photopigment in proportions roughly 3 : 1. Except for certain differences in the temperature characteristics of retinene$_1$ formation, there is no important distinction between this pigment and vertebrate rhodopsin. We propose therefore to refer to it simply as rhodopsin. It is the first light-sensitive pigment to be demonstrated directly in an invertebrate eye.

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[1] This investigation was supported in part by a grant from the Medical Sciences Division of the Office of Naval Research.

An experimental method for rapid determination of extra-polar potential distribution in nerve. PETER A. STEWART.

Since slow changes leading eventually to the complete disintegration of a dissected nerve always occur, it is essential that data be taken rapidly from such preparations. Fortunately the data needed for studies of effective membrane impedance are well suited to rapid determination.

Simple circuit analysis indicates that under sub-threshold sinusoidal stimulation the effective conductance and susceptance of the membrane at any one frequency are determined by α, the exponential rate of decay of the amplitude, and β, the linear rate of phase change with distance of the extra-polar voltage. The susceptance is proportional to the product of these factors, the conductance to the difference of their squares, the proportionality factor being the longitudinal resistance per centimeter of the nerve.

To determine α and β, the nerve is mounted vertically in four ring electrodes in a moist chamber. One pair of electrodes is used as pick-up electrodes, one of which moves along the nerve; the other pair as stimulating electrodes. The voltage at the movable electrode is amplified and applied to the vertical deflection plates of an oscilloscope through a special exponentially tapered potentiometer. The rotation of this potentiometer drives a pen along the ordinate of a sheet of graph paper, which is moved laterally in synchronism with the motion along the nerve of the pick-up electrode. Turning the potentiometer to keep the amplitude of the oscilloscope pattern constant as the pick-up electrode moves yields a straight line plot of the logarithm of voltage against distance, the slope of which is α.

A sinusoidal voltage of variable phase is used as a horizontal deflection voltage on the oscilloscope. By varying this phase to keep the shape of the pattern constant, a pen driven by the phase control knob draws a straight line plot of phase against distance, the slope of which is β.

It is found to be possible to draw such a pair of curves in about 30 seconds.

The significance of the periblast in epiboly of the Fundulus egg. J. P. TRINKAUS.

The significance of the periblast layer in the early development of the Teleost egg has long been a matter of speculation. An experimental approach to the problem, however, is clearly to be desired. The present investigation constitutes such an approach. It is concerned with the role of the periblast in the morphogenetic movements of epiboly in the eggs of *Fundulus heteroclitus.*

Although the blastoderm as a whole clings rather closely to its underlying syncytial periblast, invaginated cells of the embryonic shield particularly tending to adhere to it, the only actual connection of the two layers is at the margin of the blastoderm. Here the blastoderm is connected to the periblast by means of its thin outer epiblast layer ("Deckschicht") and its accompanying surface gel layer. By severing this connection in blastula and gastrula stages, the periblast may be entirely freed of the blastoderm and its subsequent development observed.

(It may be noted, incidentally, that a technique is hereby available for culturing blastoderms which are truly yolk-free, as well as periblast-free. These would constitute excellent material for an analysis of the role of the yolk and periblast in embryonic differentiation.)

After removal of the blastoderm, such an exposed periblast will completely encompass the yolk sphere in an epibolic movement that proceeds at about the same rate as in normal controls. In the course of this epiboly the periblast layer becomes thinner and the nuclei more widely dispersed, as in the periblast of intact eggs. These facts and the results of carbon marking experiments on exposed periblasts indicate that there is a general expansion of the periblast in the course of epiboly.

When only the central area of the blastoderm is removed in late blastula or gastrula stages, and the marginal cells (e.g., germ ring) are left attached to the periblast, epiboly proceeds at close to normal rate. The margin of the blastoderm is thus carried vegetally resulting in a bunching of cells at "closure of the blastopore."

These experiments suggest that epiboly in the intact Fundulus egg is probably not due to an autonymous expansion of the blastoderm. Attention is directed rather to the activities of the periblast, whose epibolic tendencies may be an important factor in initiating and controlling the epiboly of the overlying blastoderm.

The behavior of the surface gel layer of the Fundulus egg during epiboly. J. P. Trinkaus and Rosemary Gilmartin.

The behavior of the surface gel layer in epiboly was studied by the method of carbon marking, the fate of the adhering carbon particles being followed by means of camera lucida tracings. Eggs of *Fundulus heteroclitus* served as material for the investigation.

A carbon particle, adhering to the surface gel layer of the yolk sphere vegetal to the blastoderm, is approached during epiboly by the advancing margin of the blastoderm. When reached by the edge of the marginal periblast, it remains at this point and is carried vegetally during the continuing epiboly. A mark deliberately placed on the margin of the periblast behaves in a similar fashion. This is true for both intact eggs and eggs in which the periblast had previously been completely exposed by removal of the entire blastoderm (see Trinkaus, 1949, *Biol. Bull.*). A mark on the margin of the blastoderm itself adheres to this point during the course of epiboly in a similar fashion.

As the blastoderm envelops the yolk in epiboly, the surface gel layer of the yolk sphere continually decreases in area until with closure of the blastopore it disappears. A question arises, therefore, as to the fate of the surface gel layer of the yolk sphere. Does it contribute to the surface gel layer of the blastoderm and thus become underlain by the epiblast cells of the advancing blastoderm; or does it contribute to the surface of the expanding periblast? These marking experiments do not support either possibility. They suggest rather that the surface gel layer of the yolk sphere somehow disappears from the surface at or in the immediate vicinity of the margin of the periblast. This occurs in such a manner, however, as not to disrupt the connection between the surface of the periblast and the surface gel layer of the yolk. Exactly how this takes place is, of course, open to conjecture. These data, furthermore, support the conclusion that epiboly of both the cellular blastoderm and the underlying syncytial periblast entails an expression of material already present in these areas at the onset of gastrulation.

A preliminary study of the factors influencing the distribution of bottom fauna in two narrow arms of Buzzards Bay. George C. Whiteley, Jr., William D. Burbanck, and Madelene E. Pierce.

Study of the bottom organisms of Rand's Harbor, selected for an experiment in fertilizing small arms of the sea, was carried out in 1946, 1948, 1949. The 1949 series of transects is the most complete one so far.

During 1946 when extensive salinity records were kept, salinities at the surface varied considerably from June to September, yet bottom salinities varied only from 27–29 parts per thousand. There is a large fresh water inflow to each arm and a tidal exchange [1] of at least 50 per cent of the total volume of the arms.

During 1949 in the large arm, 67 sampling stations along 8 transects were studied; in the small arm, 53 sampling stations along 7 transects were studied. All samples, except at high and low tide, were collected from a boat by means of a modified Ekman dredge 482 sq. cm. in area, or by a small orange peel dredge of approximately the same area.

Forty-three species of invertebrates including the locally rare *Cyathura carinata* were identified. Of these 11 genera, namely, Cistenides, Clymenella, Glycera, Neanthes, Haploscoloplos, Lumbrinereis, Mya, Mulinia, Nassa, Venus, and Uca, occurred in at least 50 per cent of the transects.

Distribution of the species most frequently occurring in 1949 was similar to that of 1948 and 1946, with the exception of *Cistenides gouldi* which was 200 times more numerous in 1948.

A comparison according to number of species found at different depth zones showed that the zones of low tide and slope not only supported the greatest diversity of species but also the largest number of individuals. Distribution according to substrate showed that sandy mud and sand and organic material were preferred substrates.

The mud snail, *Nassa obsoleta*, is the most characteristic animal of this harbor.

It is believed that variations in bottom temperatures and bottom salinities are among the least critical factors influencing distribution. Turbidity due to tidal and current action may interfere with normal feeding of the filter feeders.

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[1] Data supplied by Albert Rosenburg.

The particle status of proteins. Dorothy Wrinch.

The Sorensen view that many native proteins are systems of dissociable components has long been held. Today physicochemical evidence and evidence from the more precise studies in protein crystallography are accumulating which indicate that some even relatively small proteins are particles rather than single chemical entities.

The early x-ray studies of dry crystalline insulin (Crowfoot, *Chem. Rev.* **28**: 215, 1941) uncovered a trigonal structure of molecular weight ca. 36,000. Physicochemical investigations (Oncley) show that, at pH sufficiently acid, this structure dissociates into thirds and a preliminary x-ray study (Low, Cold Spring Harbor Sym. 1949) indicates that these subunits are identical. Thus the 36,000 structure may be pictured as 3 identical subunits on a 3-fold axis. The low pH required for dissociation suggests that the subunits are held together by hydrogen bond circuits of end groups on glutamine or asparagine substituents, after the manner of the acetamide crystal.

It has long been known that mild treatments, such as dilution, dissociate horse hemoglobin of molecular weight ca. 66,700 into 2 subunits and x-ray studies (Crowfoot, loc. cit.) indicate that these subunits are identical and are arranged on a 2-fold axis. Now the results of an x-ray study (Kendrew, *Acta Cryst.* 1, Dec. 1948) of horse and whale myoglobin, both with molecular weight ca. 16,700, raise the possibility that the subunits of horse hemoglobin may also be particles. It is interesting that a comprehensive amino acid analysis of whale myoglobin (Schmid, *Nature* **153**: 481, 1949) puts the residue number at 147, close to one quarter of 576, the number suggested for the hemoglobins (Wrinch, *Biol. Bull.* **95**: 247, 1948). It may be that the hemoglobins comprise 8 subunits of ca. 72 residues a piece, or 12 subunits of ca. 48 residues a piece, with the myoglobins comprising 2, or 3, such subunits. These would be compatible with C_1 skeletons, with 8 hexapeptides on t^+ faces, 8 also on t^- faces and 6 tetrapeptides on cube faces, or with one set of hexapeptides or the set of tetrapeptides deleted (Wrinch, *Science* **107**: 445, 1948). The finding that there is one free (valyl) α-NH_2 in whale myoglobin (Schmid), one free (glycyl) α-NH_2 in horse myoglobin, 6 free (valyl) α-NH_2 in horse hemoglobin (Porter and Sanger, *Biochem. Journ.* **42**: 287, 1948) does not imply that the structures are 1, or 6, "chains," but only that there are 1, or 6, residues or peptides functioning as substituents, locked into the skeletons by a single terminal (Wrinch, *Wallerstein Communications* **11**: 175, 1948).

Even these two examples show the crucial issues raised by a particle as opposed to a molecular status for proteins.

So far there seems no escape from the view that the protein essence resides in a few, maybe only a single, characteristic type of skeleton, which is stabilized by associations of the substituents emerging therefrom. The finding that "proteins" occur in a vast variety of sizes and shapes does not conflict with this view, once it is interpreted as in general referring to particles and not to molecules.

With the specificity of individual proteins located in the nature, arrangement and association of the R-groups, a particle will have a specificity dependent on the spatial pattern of its molecules. This means that mild treatments, leading to gross changes in size and shape, may effect changes in biological specificity. Such phenomena are to be distinguished from the entirely different type of change in a protein, often called "denaturation," which on the present view result from disjoining of the skeleton and loss of protein status.

(This work is supported by the Office of Naval Research under contract N8onr-579.)

Experiment studies on the heat regulation arctic and tropical warm blooded animals. P. F. Scholander. No abstract submitted.

The uptake and loss of K^{42} in the unfertilized and fertilized eggs of Strongylocentrotus purpuratus and Arbacia punctulata. Edward L. Chambers.[1]

Carrier free K^{42} was added to 0.2 per cent suspensions of unfertilized and fertilized eggs and the penetration of K^{42} into the eggs measured by the method already described (*Biol. Bull.* **95**: 252 and 262, 1948). After a sufficient quantity of K^{42} had entered the unfertilized eggs, a portion of the suspension was removed, the eggs centrifuged down and washed twice with sea water. Two suspensions of these washed eggs were prepared and one lot fertilized. The loss of K^{42} from these washed eggs was measured by removing samples at intervals of time and determining the radioactivity remaining in the eggs.

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[1] Under grant from the NCI, USPHS. University of California at Berkeley, New York University, and the Eli Lilly Research Laboratories, M.B.L., Woods Hole, Mass.

In the uptake experiments the specific activity of the fertilized eggs (a_i/C_i) reaches 85 to 100 per cent of the specific activity of the surrounding sea water (a_0/C_0) after a period of 15 to 20 hours. The specific activity of the unfertilized eggs slowly rises to 20 per cent of the sea water after 15 hours. After this time the eggs lose their fertilizability.

The fraction of total in the eggs exchanged per minute (k) was calculated from the K^{42} uptake data using the following equation, adapted from Krogh, 1946:

$$k_{(\text{uptake})} = \frac{1}{t} \ln \frac{1}{\left(1 - \dfrac{a_i}{a_0} \dfrac{c_0}{c_i}\right)},$$

and from the K^{42} loss data, using the equation:

$$k_{(\text{loss})} = \frac{1}{t} \ln \frac{1}{(a_i/a_{i_0})}.$$

In these equations, t = time in minutes after adding K^{42} to the suspension in the case of the uptake experiments, or after washing the eggs free of K^{42} in the loss experiments, $a_i[K^{42}]$/ml. eggs, $a_{i_0}[K^{42}]$/ml. eggs at 0 time, C_i mM K^{39}/ml. eggs, C_0 mM K^{39}/ml. sea water.

The plots of $\ln \left(1 - \dfrac{a_i}{a_0} \cdot \dfrac{c_0}{c_i}\right)$ and of $\ln \left(\dfrac{a_i}{a_{i_0}}\right)$ against t are linear. Very dilute suspensions of eggs were used in order that the above simplified equations could be used. The results obtained in two typical experiments on the eggs of *S. purpuratus* are presented in the accompanying table:

	$k_{(\text{uptake})}$	$k_{(\text{loss})}$
Unfertilized	.00022	.00053
Fertilized	.035	.083
$\dfrac{k_{(\text{fertilized})}}{k_{(\text{unfertilized})}}$	16	16

The fraction of total K exchanged per minute calculated from the data on the loss of K^{42} was approximately twice that calculated from the data on the uptake of K^{42} (see table). This indicates that all the K within the egg does not exchange with the exterior at the same rate. However, on the basis of the calculations of both the uptake and loss experiments, the $k_{(\text{fertilized})}$ values are 16 times the $k_{(\text{unfertilized})}$ values (see table). The overall rate of K exchange in the fertilized eggs is, therefore, 16 times faster than in the unfertilized eggs.

The glycogen content of some invertebrate nerves. WILLIAM SCHALLEK.

Holmes (*Biochem. J.* **23**: 1182, 1929) observed that nerves of the crab Cancer had a glycogen concentration many times greater than that of mammalian nerve. It seemed desirable to repeat and extend these observations, using improved methods of glycogen assay (Kerr, *J. Biol. Chem.* **116**: 1, 1936). Glycogen and galacto-lipids were removed from the tissue with alcoholic KOH; the lipids were then removed with a methyl alcohol-chloroform mixture. The glycogen was hydrolyzed in HCl; the resultant glucose was assayed with a photo-electric colorimeter, using the copper reagent of Somogyi (*ibid.* **160**: 61, 1945) and the color reagent of Nelson (*ibid.* **153**: 375, 1944). The data is presented as mg. glycogen per 100 g. fresh weight of tissue. A and B are separate experiments, performed on different animals on different days.

	A	B	
Spider crab, *Libinia emarginata*			
Leg nerve	1680	1545	mg. per cent
Ganglion	1480	1180	

	A	B	
Squid, *Loligo pealei*			
Stellate ganglion	463	436	mg. per cent
Optic ganglion	259	236	
Cerebral ganglion	232	226	
Stellar nerve	58.5	39.8	
Axoplasm	< 1	< 1	
Remainder of nerve	21.7	20.1	

For comparison, the following data on mammalian nerve may be quoted: Rabbit nerve, 48 mg. per cent, Holmes et al., *Am. J. Physiol.* **93**: 342, 1930. Rabbit brain, 82 mg. per cent, Kerr and Ghantus, *J. Biol. Chem.* **116**: 9, 1936.

The amount of glycogen in the squid ganglia is intermediate in value between that of rabbit brain and the crab ganglion. Squid nerve, on the other hand, has practically the same glycogen content as rabbit nerve; crab nerve differs markedly in having a concentration approximately 30 times as great.

The axoplasm was separated from the remainder of the squid nerve by extrusion into distilled water. Here it formed a cylindrical clot, which was promptly transferred to KOH. No detectable glycogen was found in the axoplasm; all the content was in the remainder of the nerve. The concentration in the remainder should therefore be greater than that in whole nerve; this does not appear in the data, apparently because the determinations on whole nerve were made on different animals some time before the determinations in which the axoplasm was extruded. The distribution of glycogen outside the axoplasm accords with that reported for cholinesterase and adenosinetriphosphatase, but not with that for succinic dehydrogenase and cytochrome oxidase (Nachmansohn et al., *J. Neurophysiol.* **4**: 348, 1941; **5**: 109, 1942; **6**: 203, 1943; Libet, *Biol. Bull.* **95**: 277, 1948). Havet has reported that glycogen is concentrated in the neuroglia rather than in the neurons (*Cellule* **46**: 179, 1937).

PAPERS READ BY TITLE

Description of and lipid localization in cells of body cavity fluid of Arenicola marina (Lamarck) and Amphitrite ornata (Leidy). Ruth P. Alscher.

Romieu (*Archives de Morphologie Générale et Expérimentale* **17**: 1923) described the cells found in the body cavity fluid of *Amphitrite Edwardii, Amphitrite rubra* and some of the cells from *Arenicola marina.*

More detailed studies of the cells in the body cavity fluid of *Arenicola marina* and another species, *Amphitrite ornata,* were made employing modern techniques. The body cavity fluid from *Arenicola marina* and *Amphitrite ornata* was obtained with the aid of a hypodermic needle and syringe. Microscopical observations of various samples of fresh body cavity fluids of these marine annelids were made.

It was observed that the fluid from *Arenicola marina* contains 5 different types of cells of which 2 types are sexual cells: (1) small, mononucleated cells with heterogeneously granular cytoplasm; (2) large, mononucleated cells with lobes of densely granular cytoplasm; (3) small, amoeboid cells; (4) egg cells; (5) sperm cells.

The body cavity fluid from *Amphitrite ornata* contains 8 different types of cells of which 2 types are sexual cells: (1) large, anucleated cells with red pigment granules; (2) large, oval, mononucleated cells with heterogeneously granular cytoplasm; (3) small, oval, anucleated cells with densely granular cytoplasm; (4) small, anucleated cells with clear cytoplasm; (5) small, mononucleated cells with yellow, homogeneous cytoplasm; (6) large, anucleated, vacuolar cells; (7) egg cells; (8) sperm cells.

Measurements of 50 cells of each type were made. See Table I.

The localization of lipids in these cells was studied from smear preparations of the body cavity fluids, and both the Telford Govan (*J. Path. Bact.* **56**: 262–264, 1944) and Jackson (*Onderstepoort J. Vet. Sci. Animal Ind.* **19**: 169–177, 1944) procedures for the testing of lipids with slight modifications in technique were made. It was observed that Jackson's procedure

TABLE I

Species	Types of cells	Length of cells	Width of cells	Number of droplets of lipids per cell
Arenicola marina	1. Small, mononulceated cells	11.55μ–26.4μ	11.55μ–26.4μ	4–12
	2. Large, mononucleated cells	23.1μ–49.5μ	23.1μ–46.2μ	21–28
	3. Small, amoeboid cells	6.6μ–23.1μ	4.95μ–13.2μ	2–5
	4. Egg cells	56.1μ–231μ	56.1μ–231μ	14–43
	5. Sperm cells	The cells are visible, but they are too small to measure accurately		1–2
Amphitrite ornata	1. Large, anucleated cells with red pigment	42.9μ–121.55μ	42.9μ–121.55μ	4–38
	2. Large, oval, mononucleated cells	24.75μ–49.5μ	13.2μ–33μ	4–29
	3. Small, oval, anucleated cells with densely granular cytoplasm	13.2μ–18.15μ	6.6μ–18.15μ	1–10
	4. Small, anucleated cells with clear cytoplasm	11.55μ–82.5μ	11.55μ–74.25μ	2–3
	5. Small, oval, mononucleated cells with yellow, homogeneous cytoplasm	9.9μ–33μ	8.25μ–33μ	2–26
	6. Large, anucleated, vacuolar cells	28.05μ–33μ	23.1μ–33μ	21
	7. Egg cells	128.7μ–269.8μ	114.4μ–255.6μ	19–92

was more satisfactory for these tissues because the lipids were stained a more brilliant and clearer red.

With the aid of camera lucida drawings the size and distribution of the lipid globules were studied. In most cells, the size of the droplets of lipids ranged from small to very large. The droplets of lipids were located at various depths throughout the cytoplasm of all the cells. An interesting fact to be noted is that the number of droplets of lipids varied in the different types of cells from the 2 worms (Table I). The egg cells in both species have the greatest number of droplets of lipids, and the larger cells have more droplets of lipids than do the smaller cells. This indicates that a possible correlation between cell size and lipid content can be made, i.e., the larger the cell the greater is the lipid content.

Detection of physiological mutants in Neurospora without the use of selective media.[1]

K. C. ATWOOD.

In Neurospora the mycelia of two different mutants, neither of which can grow on minimal medium, will readily fuse to give a mycelium which grows on minimal medium and contains a mixture of the nuclei of the two mutant strains in a common cytoplasm. Such a heterokaryon can grow on minimal medium because the nuclei of each mutant component can perform the function which cannot be carried out by nuclei of the other component.

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[1] This work was supported in part by a grant from the American Cancer Society administered by Prof. Francis J. Ryan of Columbia University.

This system has been adapted to the detection of new mutants as follows: A heterokaryon is made between any biochemical mutant, X-less, and a morphological mutant, No. 422. By itself No. 422 grows as a mass of coherent spheres, and X-less will not grow unless substance X is provided. The heterokaryon, however, has normal morphology, and grows on minimal medium. Macroconidia having several nuclei per conidium are produced by the heterokaryon, and three classes of conidia can be distinguished by plating, using the sorbose technique. Those conidia containing all X-less nuclei give rise to colonies of normal morphology which appear only if substance X is present, and those containing only No. 422 nuclei give colonies having the morphology of the No. 422 mutant. Those containing both X-less and No. 422 nuclei give normal colonies appearing on minimal plates, and when conidia of cultures established from this type of colony are plated, the three classes may be distinguished as before. However, if such colonies are isolated following treatment of the conidia with a mutagenic agent, and the conidia of the resulting cultures plated again on minimal medium, some of the plates show no colonies with No. 422 morphology. In these cases, the conidia containing only No. 422 nuclei have failed to form colonies because in some the treated conidia which contained nuclei of both types, mutations were induced in the No. 422 nuclei. A mutation occurring here, while it does not affect the growth of the heterokaryotic culture arising from a treated conidium, precludes growth on minimal medium of the No. 422 component when nuclei of this component are isolated from the other nuclei during the formation of conidia by the heterokaryotic culture.

With this method the variety of mutants obtained is not restricted by the usual limitations of selective media, since the medium which supports the growth of the mutant nuclei is the living cytoplasm itself.

Ribonucleinase activity in the development of the sea urchin, Arbacia punctulata. MAURICE H. BERNSTEIN.

The behavior of RNA during development has been elaborately described. This behavior must depend on underlying enzyme mechanisms. It was, therefore, considered important to examine the level of ribonucleinase activity during the early period of development of the sea urchin.

Estimations of activity in the eggs and the early embryos of *Arbacia punctulata* were made according to the manometric methods described by Bain and Rusch (*J. B. C.* **153**: 659, 1944). Eggs or embryos were homogenized in a glass homogenizer and put into a bicarbonate medium containing RNA as a substrate. The evolution of CO_2 was followed manometrically in a conventional Warburg apparatus. This method is based on the known splitting action of the enzyme on RNA; the acid groups liberated by this action drive off CO_2 from the bicarbonate medium; hence, CO_2 liberation is a measure of the enzyme activity.

A sharp drop in the activity of ribonucleinase was found at or shortly after fertilization. This lower level of activity was then maintained during the first twenty hours of development, which was as long as any of the embryos were cultured. Schmidt, Hecht, and Thannhauser (*J. G. P.*, **31**: 203, 1948) found the DNA content of Arbacia eggs rose sharply during the first twenty-four hours of development, while the RNA content remained constant. Mazia et al. (*Biol. Bull.* **95**: 250, 1948) have shown that the level of desoxyribonuclease activity does not change in this early period. These and other data support the idea that RNA is not a precursor of DNA.

Effect of redox dyes on development of marine eggs. MATILDA M. BROOKS.

These investigations are a continuation of studies on the mechanism of fertilization of marine eggs as related to redox potential. The methods used are described in a previous paper.[1] *Chaetopterus pergamentacea* and *Amphitrite ornata* were used. Unfertilized eggs were placed in sea water containing 2×10^{-5} per cent of one of the series of redox dyes for varying periods of time from ½ minute to 6 hours, and then transferred to sea water alone. The redox potential of the unfertilized eggs and sperm, respectively, were measured on the Coleman electrometer as previously described.

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[1] M. M. Brooks, *Growth* X, 391, 1946.

It has been shown by Lillie[1] and others that these eggs form irregular development as amoeboid forms apparently spontaneously. A few such forms were always found in the controls in spite of careful handling. However, when the eggs were subjected to a dye low in the redox scale, such as neutral red, there was about 90 per cent development in Chaetopterus including two many-celled stages, the majority as amoeboid forms with or without membranes and several swimming blastulae, which did not develop further.

In the case of Amphitrite, amoeboid forms were always in the controls, but at least three times as many when indigo tetra sulfonate dye in sea water was used.

In these preliminary experiments, it shows again the influence of an appropriate redox potential in causing development in marine eggs.

Inactivation of Cypridina luciferase by heat. AURIN M. CHASE.

Luciferase solutions from the crustacean, *Cypridina hilgendorfii,* were subjected to temperatures from 40° to 60° C. for times up to 24 hours. After the desired exposures samples were cooled rapidly to 25°, luciferin (purified by Anderson's procedure; *J. Gen. Physiol.*, 1935) was added and the luminescent reaction measured. The resulting first order velocity constants were expressed in terms of that for the reaction catalyzed by untreated enzyme.

Above 50° luciferase activity practically disappears in 15 minutes. Between 40° and 50° a rapid initial activity loss occurs during about one hour. The rate of loss then decreases abruptly, remaining relatively low. The equation for a simple first order reaction does not describe the data; rather, a mechanism involving at least two steps is indicated.

Upon cooling enzyme solutions to 25° after 15 minutes at 48°, about half of the lost activity is regained asymptotically during four hours indicating a reversible effect of temperature.

The experiments for temperatures from 40° to 50° can be described by an equation based on the following assumed mechanism:

$$N \text{ (active)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} I_1 \text{ (inactive)} \xrightarrow{k_3} I_2 \text{ (inactive)}.$$

N represents the original native form of the luciferase. I_1 is an unstable inactive form. I_2, irreversibly produced from I_1, is also inactive. k_1, k_2 and k_3 represent the velocity constants of the reactions indicated. Experiments with certain other enzyme systems have indicated a similar mechanism (e.g., Herriott, *J. Gen. Physiol.*, 1948; Kunitz, *ibid.*, 1947).

For luciferase, analysis shows k_1 and k_2 to be about 100 times as great as k_3. k_1 increases with temperature to yield an experimental activation energy of about 50,000 calories indicating protein denaturation. The corresponding value for k_3 is about 30,000. k_2 is apparently independent of the temperature, at least from 40° to 50°, but this result may be illusory.

Gross morphological effects of low temperature on the fertilized eggs of Chaetopterus. DONALD P. COSTELLO AND CATHERINE HENLEY.[2]

At the time of insemination the normal Chaetopterus egg is at the metaphase of the first maturation division. In order to study the effects of low temperature on the processes of maturation and early cleavage, experiments have been conducted involving exposure of fertilized eggs to temperatures of 2 to 5 degrees C. beginning immediately after insemination. The duration of treatment ranged from 30 minutes to 12 hours, but the observed effects were most striking after a treatment of 5 hours or longer.

The process of polar-body extrusion is completely inhibited in these cold-treated eggs. With the gradual rise to room temperature, development is resumed; the eggs round up and lose the irregular flattened shape which is observed during the exposure to cold. Within 30 minutes after the end of the treatment, there is a very exaggerated elevation of the fertilization membrane (normally quite inconspicuous in the Chaetopterus egg), similar to that observed in the egg of Nereis after treatment with alkaline sodium chloride (pH 10.5). Treatment with alkali does not produce accentuation of membrane elevation in normal fertilized Chaetopterus eggs.

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[1] F. R. Lillie, *Archiv f. Entwickelungsmechanik der Organismen* XIV, 477, 1902.

[2] Aided by grants from the American Philosophical Society and the Carnegie Research Fund of the University of North Carolina.

Cleavage begins shortly after membrane elevation; the "pear" and polar lobe stages characteristic of the normal egg are not usually recognizable. The daughter-cells resulting from the first cleavage are often equal in size; subsequent cleavages are usually very irregular and difficult to study in living material. Numerous small blebs occur over the surface of the egg before and during cleavage, and these blebs may be so pronounced as to simulate cytolysis. However, the division process continues and very atypical larvae result. No readily identifiable double embryos were observed, but exceptionally large larvae (presumably fusion products) and exogastrulae occur frequently.

Cytological studies in Nymphaea L. A. ORVILLE DAHL.

Flower bud material of the white water lily (*Nymphaea odorata* Ait.) was collected at Hyannis, Mass., for an ontogenetic analysis of its highly distinctive ring-furrowed pollen grain. Material was preserved in ⅓ propionic acid-ethanol and in Karpechenko's variation of Navashin's fixative. The meiotic condition of the anthers of various ages was determined from acetocarmine preparations. This survey indicates that the colony of plants is of interest in deviating in its chromosome number from that already published for this species. A gametic number of 42 was reported by Langlet in 1927 on the basis of other material. The collection from Hyannis has a gametic number of 28. Within the genus this number has been observed only in the new world *N. mexicana* Zucc. and one old world species (*N. Lotus* L., including "N. rubra Roxb."). At metaphase and late anaphase II (polar views), the length of the chromosomes is somewhat less than 1 micron. Starch grains are so abundant in the cytoplasm that observation of chromosomes and spindle substance is frequently rendered difficult. This was particularly the case in division II of meiosis where the relatively small spindles are largely enveloped in starch.

A larger sampling of the species throughout its range is needed for proper evaluation of any differentiation in the constitution of the chromosomal complement.

Fertilizin from the eggs of the clam, Mactra solidissima. CHARLES B. METZ AND JOANNE E. DONOVAN.[1]

The view that fertilizin plays a significant role in fertilization has been questioned on the grounds that fertilizin has not been shown to occur universally. Therefore, it is of interest to report the presence of this sperm isoagglutinin in the egg water (supernatant sea water of egg suspensions) of another form, the clam *Mactra solidissima*. Mactra fertilizin was first detected by its effect on the fertilizing power of homologous sperm: Mactra egg water markedly reduced the fertilizing power of Mactra sperm. This inhibitory effect was ascribed to fertilizin because Mactra blood lacked this property. Since no agglutination of homologous sperm by Mactra egg water was observed in early tests, it was at first assumed that natural Mactra fertilizin existed only in a non-agglutinating univalent form. More recently, however, strong fertilizin preparations have been obtained which have pronounced agglutinating action on Mactra sperm. Therefore Mactra fertilizin can exist in multivalent form. Strong fertilizin preparations appeared to have a moderate activating as well as agglutinating effect on homologous sperm. The sperm agglutinated tail to tail, head to head and probably head to tail. The agglutination did not reverse appreciably on standing for one hour. Mactra fertilizin was specific to the extent that it did not agglutinate Echinarachnius or Arbacia sperm.

Adenosine triphosphate and the luminescence of the "railroad worm" and other luminous organisms. E. NEWTON HARVEY.

McElroy's (*Proc. Nat. Ac. Dc.* 33: 342, 1947) demonstration of the striking action of adenosine triphosphate (ATP) in continuing the luminescence of extracts of fire-fly lanterns has raised the question as to how general the ATP action is on other groups of luminous animals. Negative results have been obtained by Chase and McElroy with extracts of the dried ostracod crustacean, Cypridina, and by McElroy on luminous bacterial extracts. Recently Dr. P. Sawaya of São Paulo, Brazil, has sent me a specimen of the rare luminous beetle, Phrixothrix or "railroad worm," making it possible to test ATP on both red and yellow luminous organs. The ATP solution was prepared in the usual manner and shown to be active with a fire-fly (Photinus) lantern extract. No red luminiscence was obtained on adding ATP to a water extract of the red luminous organ of Phrixothrix and also none with the yellow luminescent organ. The latter result is not regarded as conclusive because the yellow light organs are very small and cannot be removed without much nonluminous tissue, thus making the extract highly dilute. The red light organ extract was concentrated and the experiment appears satisfactory. No luminescence was obtained on adding ATP solution to extracts of the luminous ctenophore, Mnemiopsis, or to extracts of the luminous annelid, Chaetopterus, under conditions regarded as satisfactory for demonstrating an ATP effect.

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[1] Aided by a grant from the National Institute of Health, U. S. Public Health Service.

Cytological effects of low temperature on the fertilized eggs of Chaetopterus. Catherine Henley and Donald P. Costello.[1]

A preliminary cytological study has been made of fertilized Chaetopterus eggs treated with low temperature, as described in the preceding abstract. Whole-mount Feulgen preparations were used chiefly; these were stained according to a method described by Anna R. Whiting (in press). A few additional preparations were made by the flattening method (Tyler, 1946). Treated and control eggs were fixed in Kahle's and Bouin's fluids at various intervals in order to secure samples of as many division stages as possible.

Polar bodies were not present in the great majority of treated eggs; at the end of the treatment 9 tetrads were clearly visible on each maturation spindle. These tetrad configurations were considerably more diffuse and complex than the normal ones.

A wide variety of abnormal mitotic configurations was observed in preparations fixed at the time of first and later cleavages in the treated eggs. These abnormalities included multipolar spindles, anaphase and telophase spindles with lagging and "lost" chromosomes or chromosome fragments, and masses of relatively uncondensed chromatin which apparently represented abortive prophase figures. In general, the types of mitotic anomalies observed were strikingly similar to those reported as occurring in the epithelium of larval salamanders exposed to low temperature. Multipolar figures were observed in a few of the control eggs; these are presumed to result from polyspermy. Since more than one sperm nucleus is often visible in the cold-treated eggs, the multipolar spindles in the experimental eggs may be the result of polyspermy facilitated by the cold treatment, rather than a direct effect of the low temperature.

Although extensive chromosome counts have not yet been completed, the available evidence indicates that at least some of these cold-treated eggs may be polyploid. Further cytological studies are in progress.

Studies of the Nereis egg jelly with the polarization microscope. Shinya Inoué.

Beneath the vitelline membrane of the *Nereis limbata* egg, there is a layer of granules which disappear upon insemination and give rise to a very thick layer of jelly around the egg. Each of these granules possesses a strong birefringence with definite axis which suggest their crystalline nature. These granules are arranged radially and are partially responsible for the radial positive birefringence of the layer in which they are distributed.

The jelly extruded from the activated egg shows a radial positive birefringence which is stronger near the egg surface. This changes into a radial negative birefringence when the jelly is compressed. Also, a shearing force induces an apparently positive birefringence in the direction parallel to the shear. The induced birefringence in both cases is stronger, the stronger the deforming force.

If the egg is treated with saturated magnesium sulphate or ammonium sulphate and subsequently returned to sea water, or treated with distilled water, the vitelline membrane is elevated and a large clear zone appears. This zone shows a radial positive birefringence which is stronger towards the central protoplasmic mass. When such an egg is compressed to different degrees, it shows proportionally strong radial negative birefringence, which returns to its original form in a few seconds after the deforming force is released. This indicates that the clear zone is filled with a gel of similar optical property as the extruded jelly.

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[1] Aided by grants from the American Philosophical Society and the Carnegie Research Fund of the University of North Carolina.

Ferry has pointed out that this jelly is a polysaccharide, and the observation above also shows that the jelly is most likely composed of a linear polymer which has a larger index of refraction along the axis of its main chain.

A Feulgen-positive reaction of the oil droplets in the Nereis egg. ROBERTA LOVELACE.[1]

The surface of the oil droplets in inseminated eggs of *Nereis limbata* reacts positively to the Feulgen reagents when the eggs are fixed in Bouin's fluid or in Allen's B-15. Washing the eggs overnight in 95 per cent alcohol removes the Feulgen-positive substances from the oil-protoplasm interface in most eggs. Eggs which have been treated with isotonic calcium chloride at 5 minutes after insemination do not lose the positively-reacting substances readily in 95 per cent alcohol. When eggs are fixed in Kahle's fluid or in Carnoy-Lebrun fixative, the positive reaction of the oil drops is less conspicuous and may be absent in some eggs. In uninseminated eggs, the oil droplets are not affected by the Feulgen reagents, even when the fixing agent contains chromic acid. The oil droplets of developing embryos and of swimming trochophores also react positively following fixation in Allen's B-15, Bouin's and Kahle's fluids. Oil droplets are unaffected by the stain in non-hydrolyzed eggs. These observations suggest that some metabolic activity which is set off in the egg by contact with the sperm results in the formation of plasmal substances at the oil-protoplasm interface. For whole mounts of Nereis eggs and embryos (using a method devised by Anna R. Whiting), fixation for 1 hour in a mixture of 8 parts of Allen's B-15 stock solution with 2 parts of 10 per cent chromic acid solution gives beautiful preparations. A sulfur dioxide water bath before staining with leuco-basic fuchsin improves the preparations.

Sulfhydryl inhibitors and a seminal fluid factor in sperm respiration. LEONARD NELSON.

It has been previously observed that sulfhydryl inhibitors in low concentrations cause increases in oxygen consumption of sea urchin spermatozoa of up to 300 per cent. Sperm used in these studies had been centrifuged once and resuspended in filtered sea water. Under these conditions, 1×10^{-4} M cadmium chloride caused a 140 per cent increase and 1×10^{-3} M $CdCl_2$ caused a 51 per cent increase in oxygen uptake. Higher concentrations inhibited respiration. However, if the "dry" sperm is centrifuged for fifteen minutes at a relative centrifugal force of 1250, the seminal fluid decanted, the sperm washed twice with filtered sea water, and finally resuspended in the first wash water, the increase in oxygen uptake in the presence of $CdCl_2$ is halved (Table I); and if the spermatozoa are resuspended in diluted seminal fluid the cadmium effect is not noted.

The QO_2 of higher densities of sperm suspension in undiluted seminal fluid (conditions approximating those within the testis) is somewhat lower than that of the sperm in sea water.

TABLE I

Sperm density	Suspending medium	$CdCl_2$ (M)	O_2 uptake (CMM)	Per cent increase
			60 min.	
5 per cent	Filtered sea water	—	34	—
5 per cent	Filtered sea water	5×10^{-5}	50	47
5 per cent	First wash water	—	37	9
5 per cent	First wash water	5×10^{-5}	41	21
5 per cent	Diluted sem. fluid	—	63	85
5 per cent	Diluted sem. fluid	5×10^{-5}	63.5	87

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[1] Aided by a grant from the Faculty Research Fund of the University of South Carolina.

TABLE II

Sperm density	Suspending medium	$CdCl_2$ (M)	O_2 uptake (CMM)	Per cent increase
			60 min.	
9 per cent	Filtered sea water	—	64	—
9 per cent	Filtered sea water	10^{-3}	104	63
9 per cent	Undiluted sem. fl.	—	56	−12.5
9 per cent	Undiluted sem. fl.	10^{-3}	87	36
9 per cent	Boiled sem. fluid	—	78	22
9 per cent	Boiled sem. fluid	10^{-3}	115	80
			120 min.	
5 per cent	Filtered sea water	—	81	—
5 per cent	Filtered sea water	10^{-3}	127	57
5 per cent	Ground glass sem. fl.	—	181	124
5 per cent	Ground glass sem. fl.	10^{-3}	140	72

Addition of $CdCl_2$ to the seminal fluid, boiling or filtering the fluid through ground glass, and even dilution in sea water, will raise the QO_2.

These data indicate the presence in seminal fluid of a factor perhaps similar to that described by Hayashi.[1] If this seminal fluid factor is likewise protein in nature, perhaps the stimulatory effect may be attributed to the inactivation of the factor by mercaptide-formation of its sulfhydryl groups by cadmium ions.

Extrusion of jelly by eggs of Nereis limbata under electrical stimulus. W. J. V. Osterhout.

Insemination causes Nereis eggs to extrude jelly. This is produced by the swelling of jelly precursor granules inside the protoplasm. In the absence of sperm extrusion of jelly can be produced by weak electrical currents, e.g., by alternating or direct currents of 17 milliamperes per cm.^{2} of cross section applied for 1 minute (W. Cattell obtained artificial parthenogenesis with much larger direct currents).

These experiments were made at approximately 15°, 20°, and 25° C.

When 17 milliamperes are applied for 1 minute the production of jelly continues for several minutes after the current is shut off. It is much more rapid with alternating than with direct current.

In direct current the jelly may appear first inside the vitelline membrane which covers the egg on the side facing the anode and then pass through the membrane into the sea water and more toward the anode.

In alternating current the jelly usually appears first on the opposite sides facing the electrodes, and extends gradually around the egg.

The action of electrical currents is readily blocked by treating the eggs with certain reagents.

These and other experiments indicate that the effects described are not due to heat developed by the current.

It may be suggested that the micelles of the jelly precursor granules migrate cataphoretically so as to promote access of water to the substance which swells to produce the jelly. If the granules have a waterproof covering as suggested by D. P. Costello this hypothesis may apply to the covering.

The influence of glycolysis on the potassium and sodium content of Saccharomyces cerevisiae and the egg of Arbacia punctulata. George T. Scott and Mary E. Rice.

Danowski (1940) has observed a loss of potassium from erythrocytes in the presence of sodium fluoride. Harris (1941) in addition demonstrated a greater loss of potassium in the cold in the absence of glucose. Wilbrandt (1940) reported a loss of potassium in the presence of iodoacetic acid. Recently Dixon (1949) has indicated a significant potassium loss from brain slices under conditions of anaerobiosis in the absence of glucose or under conditions of fluoride inhibition.

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[1] Hayashi. *Biol. Bull.* **89**: 162, 195; **90**: 177, 1946.

The present experiments consist of a study of the relation of glycolysis to the potassium and sodium distributions in the Arbacia egg and the yeast cell. The cell suspensions are placed in 100 ml. graduated conical centrifuge tubes for the duration of the experiment. The cells are then thrown down by centrifuging, and the total cell volume measured. After a dry weight is taken the material is "ashed" by boiling in distilled water for twenty minutes to release the potassium and sodium. The solution is made up to volume, cell debris removed by centrifuging, and analyzed in the flame photometer.

Under conditions of anaerobiosis, indicated by decolorization of methylene blue, for short periods of time (one to three hours) the potassium and sodium content does not change. In the presence of iodoacetate (1 : 5,000) from 25 to 40 per cent of the potassium content is lost from the cells. A systematic study of the rate of change of these two elements over a longer period of time in the presence of inhibitors is in progress. The possible prevention of fluoride and iodoacetate inhibition by triose will be investigated.

The adaptive utilization of sucrose by the ciliate, Colpidium camplum. Gerald R. Seaman and Benedict O'Malley.

A pure, sterile culture of *Colpidium campylum* was established in a medium consisting of 0.1M sucrose in Hahnert's solution by transferring 0.5 cc. of inoculum from a proteose-peptone medium to 10 cc. of the sucrose medium. When maximum growth was reached, 0.5 cc. of this culture was transferred to a second 10 cc. of sucrose medium. The procedure was repeated through 20 transfers. At that time calculations show that less than 1×10^{-5} micrograms of material had been carried over from the original peptone culture. All results presented were obtained from cultures which had undergone more than 20 transfers in the sucrose medium.

The growth rate in sucrose is very much lower than that obtained in peptone medium (Seaman, *J. Cell. Comp. Physiol.* **33**: 137). A maximum population of 16,000 organisms/cc. is reached 3 days after inoculation. The number of cells then decreases rapidly to a level of approximately 4,000 organisms/cc. and remains at this level for a period of about 14 days.

Manometric measurements show that organisms from a peptone medium do not utilize sucrose. If washed and starved cells (from the peptone medium) are incubated with sucrose for 6 hours there is no increase in oxygen uptake in the presence of sucrose, and no reducing sugars are recovered. However, if cells from the sucrose medium are incubated with 60 mg. of sucrose, after an induction period of 80 minutes there is a gradual increase of oxygen uptake which is 74 per cent above the endogenous rate at the end of 300 minutes. At this time, there is recovered 11 mg. of reducing sugars.

The absence of added nitrogen in the sucrose medium suggests that *Colpidium* is capable of fixing atmospheric nitrogen. Preliminary manometric determinations indicate that, in fact, the organisms do fix atmospheric nitrogen.

REPORT ON LALOR FELLOWSHIP RESEARCH

A study of the hydrogenase systems of green and blue-green algae. Albert Frenkel.

The capacity of various organisms to reduce carbon-dioxide and other substances by means of molecular hydrogen was investigated. It could be shown that the ability to carry out photoreduction of carbon dioxide with hydrogen is not restricted to several species of the Clorococcales among the algae, but can also be observed in at least one of the Volvocales and in two different genera of the Myxophyceae.

The organism belonging to the Volvocales which was studied was Clamydomonas Moewusii, Gerloff (isolated by Dr. L. Provasoli, and kindly supplied by Ralph Lewin). This alga possesses a very active hydrogenase and thus differs quantitatively from green algae like *Scenedesmus obliquus* which has been studied most extensively thus far. Clamydomonas grown aerobically can be adapted to carry out reduction of carbon-dioxide with hydrogen by incubating cell suspensions for thirty minutes in an atmosphere of hydrogen in the dark. In contrast to adapted suspensions of *Scenedesmus obliquus,* Chlamydomonas cells will tolerate up to 700 foot candles of unfiltered white light (Mazda bulbs) before reversion to photosynthesis will occur, whereas *Scenedesmus obliquus* will usually revert at about 100 foot candles of white light; once reversion occurs the cells have to be re-adapted again in the dark to reactivate the hydrogenase. At low partial pressures of oxygen (2–5 mm. of mercury), adapted suspensions of Chlamydomonas cells will carry out the oxyhydrogen reaction which on the basis of oxygen consumed follows the course of a first order reaction.

$$O_2 + 2H_2 = 2H_2O$$

A number of blue-green algae were also investigated as their capacity to carry out photoreduction. Two species were found which could be adapted successfully. One of these, a species of Chroococcus, has been isolated and has been grown in pure culture. It behaves very much like a unicellular species of Synechococcus studied previously. Adaptation of Chroococcus sp. to photoreduction can be accomplished after two hours of incubation in an atmosphere of hydrogen. The cells revert to photosynthesis at about 100 foot candles of white light in a similar manner as *Scenedesmus obliquus.* Their behavior differs, however, from Scenedesmus in that the blue-green alga can be re-adapted at low light intensities (20 foot candles) as long as the partial pressure of oxygen evolved by photosynthesis does not exceed approximately 1 mm. of mercury. Chroococcus sp. will also carry out the oxyhydrogen reaction in the dark.

These algae in addition to their capacity to reduce molecular oxygen in the dark and carbon dioxide in the light by means of molecular hydrogen are able to reduce other compounds with hydrogen. *Scenedesmus obliquus* will reduce quinone to hydroquinone in the dark as follows:

$$\text{Quinone} + H_{2(g)} = \text{Hydroquinone.}$$

At low concentrations of quinone (1.5×10^{-3} moles per liter), the reaction is of the first order and 95 to 100 per cent of the added quinone can be reduced in this manner.

Work is in progress to study the reduction of nitrate by means of molecular hydrogen in the dark and in the light, and to investigate intermediate compounds formed during the reduction of nitrate to ammonia.

Marine invertebrate phosphatases. C. Albert Kind.

Although many reports on the activity of vertebrate phosphatases have appeared in the literature, data on the phosphatase activity of marine invertebrate tissues is singularly lacking. Norris and Rama Rao (*J. Biol. Chem.* **108**: 783 (1935)) demonstrated "alkaline" and "acid" phosphomonoesterase activity in echinoderms and mollusks and a relationship between "alkaline" phosphatase activity and shell formation in mollusks has been postulated by Manigault (*Ann. Inst. Oceanograph.* **18**: 331 (1939)).

The present work was undertaken to provide data on the phosphatase activity of representatives of marine invertebrate phyla for which no such information is available. The tissues were homogenized with water saturated with chloroform (20 to 25 ml. solvent per gm. tissue) and allowed to stand at 5° for 20 hours. The mixtures were centrifuged and 1 ml. aliquots of the phosphatase-active supernatants were incubated at 28° for 24 hours with solutions of beta-glycerophosphate over pH range from 4.0 to 9.0. Contrary to a report by Norris these extracts are not inactivated by heating above 30° since samples incubated for 24 hours at 37.5° showed no loss in activity. Inorganic phosphate was determined by the method of Fiske and SubbaRow (*J. Biol. Chem.* **66**: 375 (1925)).

All extracts studied showed optimum activity at p.H 4.5 to 5.0, and with the exception of extracts of Porifera and the one representative of Ctenophora, at pH 8.0 to 8.5. The activities are very low in comparison with those of vertebrate tissue and thus a relatively long incubation time is advisable. There appears to be a progressive increase in "alkaline" phosphatase activity with increasing biological complexity. Also of interest is an apparent increase in the *ratio* of "alkaline" to "acid" phosphatase activities expressed as mg.P per gm. tissue. The following values for this ratio are based on two representatives of each phylum: Porifera 0.075, Coelenterata 0.29,

C. tenophora 0.75, Echinodermata 0.94, Mollusca 1.6. Any phylogenetic significance in the increase of the "alkaline" to "acid" ratio must of course be based on a much larger number of observations but the possibility of such a relationship appears to be indicated.

Ribonucleic acid at cell surfaces and its possible significance. A. I. LANSING AND T. B. ROSENTHAL.

We have previously shown by means of Celite column chromatography and Ca^{45} labelling that a portion of mouse liver calcium is bound to a ribonucleoprotein. One of our students (Li) has shown that both calcium and ribonucleic acid are lost from the region of liver cell surfaces after in vivo administration of ribonuclease. The present experiments were designed to localize the peripheral ribonucleic acid in marine eggs.

Unfertilized and fertilized eggs of Arbacia, Asterias, Chaetopterus, and Echinarachnius were exposed to ribonuclease in sea water (controls to boiled ribonuclease), fixed in alcohol-formalin, imbedded in paraffin and sectioned at 4 micra, and stained for 2 to 5 minutes in 0.5 per cent toluidine blue.

The control unfertilized eggs showed a thin band of basophilia in both the cell cortex and in the vitelline membrane; this basophilia was more pronounced in the fertilized eggs. A moderate metachromasia was noted in the vitelline and fertilization membranes. The ribonuclease treated eggs showed a complete loss of basophilia in the cell cortex and in the vitelline membranes. The fertilization membranes retained a small amount of the blue dye while the red metachromasia was quite pronounced. We have concluded that the cell cortex of these eggs contains ribonucleic acid and that the vitelline and fertilization membranes contain ribonucleic acid with a second moiety responsible for the metachromasia. The latter, which may well be a polysaccharide sulfate ester, is under further investigation.

On the basis of Neuberg's impressive experiments (*Arch. Biochem.* **20**: 185–210, 1949) which indicate that nucleic acids have a tremendous capacity for complexing with minerals, we have suggested that the ribonucleic acid at cell surfaces may be involved in the ion uptake mechanism of cells. This possibility is supported by a simple experiment on Elodea leaves.

The Elodea leaves were citrated to remove calcium, exposed to ribonuclease (controls to boiled ribonuclease) then to 0.02 M $CaCl_2$ or $SrCl_2$, plasmolyzed in 0.5 M sucrose and deplasmolyzed in pond water. This results in formation of numerous Ca and Sr oxalate crystals (cf. Mazia, *Biol. Bull.* **71**: 306–323, 1936). However, the ribonuclease treated leaves showed almost complete inhibition of crystal formation, which may be due to failure of the calcium or strontium ions to be bound.

Determination of amino acids in invertebrates. JERRE L. NOLAND.

With the recent development of the microbiological procedures, it has become relatively easy to determine amino acids quantitatively in biological materials. To date, however, there are few reliable values for the amino acid composition of invertebrate tissues. In the present project the blood and muscle of the following animals are being analyzed for 18 amino acids:

Phylum	Species	Tissue analyzed
Annelida	*Phascolosoma gouldi* (Pourtales)	coelomic fluid muscle
Mollusca	*Mactra solidissima* Dillwyn	blood muscle
	Loligo pealei Lesueur	blood mantle
	Busycon canaliculatum Say	muscle
Arthropoda	*Limulus polyphemus* L.	blood blood clot muscle
	Callinectes sapidus Rathbun	blood muscle
Echinodermata	*Thyone briareus* (Lesueur)	muscle
Chordata	*Squalus vulgaris*	blood muscle

The blood samples were diluted with distilled water, and the protein precipitated according to the method of Hier and Bergeim (*Jour. Biol. Chem.* **161**: 717 (1945)). The blood filtrate was adjusted to pH 6.8 and stored in the cold under toluene. The muscle tissue was isolated by dissection, washed in tap water, dried at 60° C. in vacuo and powdered. Aliquots of the powder were hydrolyzed with 4 N HCl or NaOH, adjusted to pH 6.8, diluted to volume and stored under toluene in the cold.

The determinations were made by a microbiological procedure, using *Leuconostoc mesenteroides* P-60 or *Leuconostoc citrovorum* 8081 grown on medium VI of Steele et al. (*Jour. Biol. Chem.* **177**: 533 (1949)). The lactic acid produced after 72 hours incubation was titrated electrometrically with 0.02 N NaOH.

Tentative values have been obtained for the amino acids tryptophan, tyrosine, histidine, leucine and proline. From these data it appears that the blood of Callinectes has the highest concentration of "free" amino acids, followed in order by Loligo, Phascolosoma and Squalus. The blood filtrates of Mactra and Limulus appeared to be practically free of these amino acids. The results of the muscle analyses indicate that invertebrate muscle must be similar in composition to muscle from vertebrates. This is in accord with the scattered data already published concerning the amino acid composition of muscle.

The release of radioactive Ca^{45} from muscle during stimulation. ARTHUR A. WOODWARD, JR.

The release of Ca^{45} from frog muscle was studied. After all attempts to get a usable amount of Ca^{45} into isolated single muscle fibers had failed, experiments were thereafter performed with whole sartorius muscles. These were carefully dissect to avoid the slightest injury and then immersed for 3 to 4 hours at 5° C. in Ringer solution containing 5 microcuries per ml. of Ca^{45}. The muscles were then removed, rinsed, and washed in successive equal portions of fresh Ringer solution; the time spent in each washing bath was the same in any given experiment, usually either 1 min. or 3 min. After 5 to 7 of such washings the muscles were then stimulated electrically while immersed for the same length of time in a final bath of Ringer solution. Each of these washings was then dried and the amount of radioactive material present determined with a Geiger-Muller counter.

The amount of Ca^{45} given off to the washing baths dropped rapidly in successive samples to a low level and then continued to decrease slowly and rather uniformly. Electrical stimulation caused increases of from 30 per cent to 200 per cent in the amount of Ca^{45} released to the bathing medium. Analysis of curves obtained from plotting the data leave little doubt that the Ca^{45} thus released came from the muscle fibers rather than from the interfibrillar spaces. This was confirmed by experiments in which the first period of stimulation was followed by alternate washings at rest and during stimulation. Stimulation always produced an increase in Ca^{45} released, and that given off during rest always dropped back to its previous level.

In other experiments carefully matched muscles were selected and exposed to increasing amounts of stimulation by increasing the frequency of stimulation over a given time interval. Increase in the amount of stimulation always produced an increase in amount of Ca^{45} released to the bathing medium. It is concluded that electrical stimulation of frog muscle causes a release of free Ca ions from the protoplasm of the muscle fibers.

Proteolytic enzymes in cytoplasmic granule preparations of Arbacia eggs. ARTHUR A. WOODWARD, JR.

Preliminary but very scant observations show the presence of proteolytic enzyme activity in preparations of cytoplasmic granules obtained from unfertilized Arbacia eggs by fractional centrifugation (partial fractionation only) after the method of Harris (1942). Only one preparation of granules suitable for satisfactory enzyme assay was obtained. This sample contained all the granular fractions of the cytoplasm, but was free of whole cells, nuclei, membranes, and other extraneous materials. The preparation had a low but significant protease activity which was increased from 35 per cent to 50 per cent upon the addition of cyanide. The addition of Ca ions to the preparation had no detectable effect on the enzyme activity. The sample was too small to admit of further fractionation of the granules with profit. The method of Anson (1938) was used for assay of proteolytic enzymes.

Electrophysiological measurements on the eyes of Limulus and Loligo. V. J. WULFF.

1. Measurement of asymmetry and action potentials.

All attempts to obtain a measurement of asymmetry potentials in the lateral eye of Limulus met with negative results. It was concluded that the orientation of an asymmetry potential, if it exists at all, must have been normal to the axis of the electrodes. This interpretation is indicated by the morphology of the sense cells and merits further investigation.

Similar measurements on the excised eye of the squid, *Loligo pealei,* yielded asymmetry potentials ranging from 5 millivolts at the beginning of an experiment to ca. zero mv. at the end of an experiment. The asymmetry potential often reversed its polarity, so that an initially negative corneal electrode would become positive, with a subsequent decline to zero. Retinal action potentials, obtained in response to constant intensity, constant duration light stimuli at fifteen minute intervals during the course of the experiment, indicated that the magnitude of the voltage wave varied with the magnitude of the asymmetry potential. Reversal of the asymmetry potential was accompanied by reversal of polarity of the action potential. The lens and outer covering of the eye ball contributed about 50 microvolts to the overall potential. Both asymmetry and action potentials decline rapidly to zero under conditions of partial or total oxygen lack.

2. Measurement of retinal action potentials and optic nerve action potentials.

Measurements on the lateral eye of Limulus are abstracted in detail elsewhere. Similar measurements on the median eye of Limulus indicated parallelism with the lateral eye. The median eye was excised completely with its nerve and introduced into an oil-filled capillary tube. The response of the sense cells of this preparation duplicated the results obtained from the lateral eye.

Similar measurements on the excised squid eye yielded negative results with respect to measurement of optic nerve activity. It was concluded that the optic nerve fibers become rapidly inactive upon removal of the eye from the organism or that the magnitude of the nerve action potentials is so small as to be undetectable with the equipment used.

3. The effect of some chemical agents on the responses of the lateral optic pathway of Limulus.

The addition of procain hydrochloride to the fluid in the rear compartment of the eye chamber (final concentration — 0.02 per cent) immediately abolished nerve activity and slowly caused a decline in phase two of the retinal response.

The addition of sea water containing excess KCl (ten times that present in normal sea water) abolished nerve activity and caused a slow decline of the retinal response, affecting first the second phase and subsequently affecting the first phase. Before the nerve activity ceased it was observed that the "silent period" in the nerve discharge following an initial rapid burst of impulses, was greatly reduced or absent. The effect of KCl was reversible.

The addition of veratrine hydrochloride (final conc. 1 part per 500,000, 1,000,000 and 2,000,000) abolished the response of the optic nerve and produced an enhancement of the retinal action potential, affecting both phases, particularly the declining limb of the second phase.

4. The effect of light adaptation of the responses of the lateral optic pathway of Limulus.

Progressively increasing light adaptation produces a reduction followed by an increase in latency of both retinal and nerve response and a gradual encroachment of the second phase upon the first phase of the retinal response.

Characteristics of electrical activity in the lateral optic pathway of Limulus. VERNER J. WULFF.

Illumination of the lateral eye of *Limulus polyphemus* elicits a potential change, the retinal action potential. The magnitude of the retinal action potential varies with the logarithm of the intensity. The retinal action potential is followed by the appearance of activity in the optic nerve. Both retinal and optic nerve responses exhibit latencies. The latency of the latter exceeds that of the former by an interval which varies inversely with the intensity and with the magnitude of the retinal response. This difference in the latencies of the retinal and optic nerve responses is called the retinal-nerve interval. In many experiments the R-N interval progressively increases as the intensity of illumination decreases; in others the R-N interval increases with decreasing intensity up to a point and then decreases upon stimulation with still lower intensities. This peculiar relation has a parallel in the retinal response. The time for the retinal response to reach its maximum, called peak-time, exhibits a similar relation to intensity. Further study revealed that the retinal response is dual in nature. A single smooth response is evident upon stimulation with low intensities. Stimulation with higher intensities elicits a second wave of potential superimposed on the first, which grows rapidly in magnitude and eventually obscures the first phase. It is suggested that this duality of the retinal response may possibly indicate two types of sense elements (for which there is no supporting evidence) or that the sense cells exhibit a dual response, analogous to peripheral nerve. The increase of the R-N interval with lowered intensity of stimulation and, consequently, with lower magnitude of the retinal response, lends support to the hypothesis that local action currents caused by the retinal potential initiate activity in the nervous elements of the optic pathway.

PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

June 24, 1949

Specificity of desoxyribonucleases and their cytochemical application.[1] Jay Barton II and Daniel Mazia.

The specificities of Desoxyribonucleases (DNases) are of special interest because of (1) the insight they may give us into the structure of DNA, and (2) their possible application to cytochemical study of DNA.

The initial observation suggesting that there may be more than one qualitatively distinct DNase was made while developing a new method for the estimation of the enzyme. This new method is based on the precipitation of high polymer nucleic acid by protamine and the spectrophotometric determination of the remaining low polymer. No good correlation could be found between the activity as measured by this method and when measured by the usual viscometric methods, even though a crystalline preparation was used. Several points of difference were found. (1) The pH optimum when measured by the method of Barton and Mazia is about 5.6; when measured by the viscosity method the optimum is about 6.9. (2) The reaction rates are of an entirely different character. The protamine method shows a reaction of the zero order, while with the viscosity method the reaction curve approaches that of a first order reaction. (3) With the same crystalline preparation (Kunitz method, prepared by Worthington Biochemical Lab.) the limiting enzyme concentration when measured by the viscosity method is reached at about 0.1 microgram per ml. This concentration is just at the lower limit of detectability by our new method. The activity measured by this method is still proportional to enzyme concentration about 10 microgram per ml. (4) When the activity of tissue homogenates is determined by both of the methods, the ratio between "viscosity" activity and "protamine" activity is found to vary. This last observation suggests that two enzymes are involved rather than multiple specificities of a single enzyme.

Kunitz's method concentrates the enzyme measured by viscosity-lowering, but two crystalline preparations that have been tested both contain a small contamination of the activity measured by the protamine method. In every experiment made thus far, the viscosity lowering is complete before protamine-soluble DNA appears.

It is suggested that the DNase measured by the protamine method is specific for the linkages primarily responsible for polymerization of nucleotides while the viscosity-lowering enzyme is specific for another class or classes of linkages responsible for structural viscosity. From this would follow a picture of the DNA macromolecules essentially similar to that of Gulland.

Initial experiments applying the two specificities to cytochemical analysis of the mode of attachment of DNA in the salivary chromosomes of Chironomus have been made. Loss of DNA from the chromosome can be attributed to the same activity as that measured by the protamine method. The conclusion that DNA may be removed from the chromosome by rupture of internucleotide linkages is understandable only in the light of what is known of the altered solubility of low-polymer DNA-protein complexes. Both the DNA and the histone are removed together, leaving a "residual" intact chromosome behind. The inference is that the attachment to the chromosome is weak enough to be broken when the size of the DNA-basic protein complex is changed.

Physiology of tracheal filling in Sciara larvae. M. L. Keister and J. B. Buck.

During each larval instar of Sciara, the air-filled tracheae become enclosed in larger, liquid-filled, coaxial tubes which are to form the tracheal system of the next instar. At moulting the old gas-filled tracheae are withdrawn and shed with the body cuticle. Three to eight minutes later, the liquid column breaks at some single point within one of the main trunks, and gas

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[1] Work supported by a grant from the American Cancer Society, recommended by the NRC Committee on Growth.

rapidly fills the entire new system. The gas does not enter by way of the spiracles. The liquid is withdrawn into the blood and tissues. Filling of the tracheae with gas will occur in a normal manner in larvae submerged in oil or water in the absence of visible gas. Gas-filling is delayed by exposure to nitrogen, carbon dioxide or carbon monoxide containing less than 0.5 per cent of oxygen, and indefinitely inhibited by completely oxygen-free gases. High concentrations of carbon monoxide in the dark do not inhibit filling. Filling is inhibited during and for some time after exposure to low temperature. Diffusion, release of hydrostatic pressure, increase in tissue osmotic pressure, and muscular activity are shown to be inadequate to explain tracheal filling. It is suggested that an aerobic metabolic process is involved.

Bioelectrical models of energy transformation in nerve. T. C. BARNES AND R. BEUTNER.

A modification of the elongated oil-cell model of bioelectrical potential previously described to the Society of General Physiology (*Biol. Bull.* **95**: 281, 1949) has openings along the tube to permit recording of the wave produced by acetylcholine at various distances away from the point of application and at various inter-electrode distances. The wave behaves like a nerve impulse between two nodes of Ranvier (Stampfli, XVII Internat. Physiol. Cong., p. 218, 1947). The recent rediscovery of nodes in fibres of the brain (Allison, *Nature* **163**: 449, 1949) suggests that our tube model applies to the electroencephalogram. The synapse is represented by a low-resistance oil in a U-tube at the end of the long tube. This U-tube synapse responds readily to acetylcholine in contrast to the axon where resin is added to duplicate insensitivity to chemical mediators. Sensitization to histamine is produced in a stationary model by adding lauryl sulfonate to triacetin on which 0.05 per cent histamine give 20 mv. negative but has no effect in absence of lauryl sulfonate. The lauryl sulfonate solution clears on addition of acetylcholine or histamine suggesting colloidal change. Mixtures of guaiacol and resin kept for several weeks gradually lose their reaction with acetylcholine (model of aging). The role of the oils is largely physical since bromobenzene gives the same effects as nitrobenzene. All nerve substances studied give disappointing results except alkaloids (acetylcholine). 0.05 per cent ATP generates only 10 mv. positive on guaiacol in contrast to 35 mv. negative produced by acetylcholine. The positivity of ATP fails to sensitize oils further to the chemical mediator.

The effect of ultraviolet on green algae and isolated chloroplasts. A. S. HOLT AND W. A. ARNOLD.

A comparative study of the effects of ultraviolet (2537 Å) on various measurable processes occurring in algae and in chloroplast preparations shows wide variations in sensitivities. With four day old cultures of *Chlorella pyrenoidosa* and *Scenedesmus* D_1, it was found that the photosynthetic mechanism of Chlorella is far more sensitive than that of Scenedesmus. Endogenous respiration in both algae is comparatively untouched as compared to photosynthesis, while the oxidation of glucose added to starved Chlorella cells is many times more sensitive than photosynthesis. In Scenedesmus the same dosage of ultraviolet light caused the same percentage inhibition of photosynthesis, of photoreduction, and of oxygen evolution from p-benzoquinone solutions. Oxygen evolution by isolated chloroplasts and chloroplast fragments is also inactivated as shown by manometric measurements with ferricyanide as the oxidant, and by dye reduction measurements with 2,6 dichlorbenzenone-3-chlorophenol. The catalase activity of chloroplasts is not affected by doses that completely inactivate oxygen evolution. Comparative studies of the effects of the poisons HCN and NH_2OH on irradiated and untreated Scenedesmus show that both poisons reduce the rate of photosynthesis of the irradiated cells to the same percentage as they do the normal cells. Survival as measured by colony formation following irradiation is far more sensitive than photosynthesis.

Chemical induction of bud formation in plant tissues. FOLKE SKOOG AND CHENG TSUI.

Results from experiments on the interaction of indoleacetic acid and phosphate in growth and organ formation in tobacco tissue cultures led to tests on the effect of adenine and its derivatives on bud formation.

It was found that adenine supplied to the nutrient medium causes bud formation in tobacco callus and stem segment cultures under conditions where controls formed none, and increased the number of buds where the controls formed a few. The number increases with concentration of adenine up to 40 mg. per l. and may reach 40 buds on a 150 mg. piece of tissue. IAA prevents bud formation, it stimulates callus growth, and induces root formation. Mixtures of the two compounds in high Ad per IAA ratios may permit formation of both buds and roots. Rel. high concentrations of both compounds cause rapid growth of the tissues without organ formation. Many other purines have a similar but less effect than adenine on bud formation.

A controlling influence of the adenine-IAA ratio on growth and organ formation has also been established in tissues from horse radish and carrot. However, the concentration ranges for activities varies with the species.

A brief summary of tissue contents and distribution of phosphates and auxin in relation to the treatments and morphological changes which they produce will be presented.

Effects of ultra-rapid freezing on mammalian erythrocytes. B. J. LUYET.

Smears of oxalated ox blood, in layers about 60 micra thick, cooled at a rate of some hundred degrees per second by immersion in liquid nitrogen, and rewarmed at a velocity of the same order by immersion in physiological saline at room temperature, showed only a slight hemolysis and furnished, upon centrifugation, a red-cell content of about 72 per cent that of normal untreated blood. When similar smears were exposed for some 20 seconds, either during cooling or during rewarming, to the crystallization temperatures (a few tens degrees below zero), hemolysis was heavy and the hematocrit readings indicated only some 4 per cent non-hemolyzed red cells. Varying the time in liquid nitrogen from 10 seconds to 2 hours did not change the percentage of surviving cells. The possibility of an exosmosis of water, during freezing through the relatively large surface areas of such small cells may account for the fact that some erythrocytes escape injury when freezing is relatively slow, and the absence of crystalline ice in all probability explains the preservation of most cells after ultra-rapid freezing; but whether the destruction of more than a fourth of the cells upon ultra-rapid freezing is caused by the incomplete prevention of ice crystals remains to be ascertained. (If equipment and material are available, a demonstration will be made of the "vitrification" of aqueous solutions, of the prevention of hemolysis in ultra-rapid freezing and of the methods used, in general, for obtaining, controlling and recording cooling or rewarming velocities up to 20.000 degrees C. per second.)

Photosynthesis and photoreduction by a species of blue-green algae. ALBERT FRENKEL, HANS GAFFRON, AND EDWIN H. BATTLEY.

A naturally occurring enrichment culture of Synechococcus sp. found on Angelica Point in Buzzard's Bay was investigated for its capacity to carry out photosynthesis and photoreduction. Under aerobic conditions the cells carried out normal photosynthesis with a photosynthetic quotient of $\Delta O_2/\Delta CO_2$ of 1.08 ± .02.

Under anaerobic conditions in the presence of hydrogen the cells can be adapted to carry out photoreduction of carbon-dioxide by means of molecular hydrogen. In general these blue-green algae behave in the same way as Scenedesmus strains in which this process was first observed among algae. Oxygen and high light intensities will reverse the adaptation reaction so that the cells will again carry out normal photosynthesis. Sodium sulfide (10^{-3} M per L.) will inhibit the adaptation reaction in the presence of hydrogen, however, when sodium sulfide is added after adaptation has taken place it will stabilize photoreduction against reversal by high light intensities. Preliminary experiments indicate that added sulfide will not disappear as long as photoreduction is carried out in the presence of molecular hydrogen. However, when hydrogen is replaced by nitrogen and sodium sulfide is added, the latter will disappear in the light with the simultaneous reduction of carbon-dioxide. For each molecule of carbon-dioxide two molecules of sulfide will be oxidized. As soon as all the sulfide is oxidized the cells will revert to normal photosynthesis. Cultures of Scenedesmus sp. adapted to photoreduction are also able to reduce carbon-dioxide with the simultaneous oxidation of sulfide in the light, in the same way as Synechococcus sp. This process may be similar to that occurring in some of the sulfur bacteria.

Riboflavin-sensitized photo-oxidations and their significance in plant physiology. ARTHUR W. GALSTON.

In the course of investigations to determine the mechanism of the light inhibition of growth of plant stems, it was discovered that riboflavin, when applied to plants in physiological concentrations, exerts a profound inhibitory effect on growth. This inhibition is manifested only in the light, and was subsequently found to be due to a riboflavin-sensitized photooxidation of the plant growth hormone, indoleacetic acid (IAA). *In vitro*, this reaction is first order with respect to disappearance of IAA. It requires oxygen, one mol of O_2 being absorbed and 1 mol of CO_2 being released per mol of IAA photoinactivated. The oxidation product is a physiologically inactive, brown-red material, with a melting point of about 150° C., and an approximate empirical formula of $C_{12}H_{10}NO$. Its structure is still under investigation.

In addition to IAA, the following compounds may undergo riboflavin sensitized photooxidations: all indole-containing compounds, histidine, methionine, various enzymes (including urease, tyrosinase and α-amylase), immune proteins and bacteriophage T6r. Lumichrome is less effective as a sensitizer than is riboflavin, and lumiflavin is not at all effective. The activity of d-amino acid oxidase of hog kidney, which contains a riboflavin-adenine dinucleotide prosthetic group is, however, unaffected by light.

Avena coleoptile tips contain about 30 micrograms of riboflavin per gram dry weight. If the diffusible auxin of *Avena* coleoptiles is gathered into agar blocks containing traces of riboflavin, this auxin is rapidly photoinactivated. It therefore seems probable that, *in vivo*, riboflavin may sensitize the photoinactivation of IAA. If this is true, then riboflavin may be involved in the phototropic response of *Avena* coleoptiles. Although several of the published action spectra for phototropism contain a double maximum suggestive of a carotinoid receptor, they do not rule out the participation of riboflavin, which has its visible absorption maximum at about 450 $m\mu$, in the region of highest phototropic effectiveness.

It is further suggested that this type of reaction may explain the often reported fact that tissue exposed to strong light has a lower auxin content than does unilluminated tissue. The implications of such reactions for plant growth are numerous.

Studies on the rigor resulting from the thawing of frozen skeletal muscle. S. V. PERRY.

When frozen frog sartorious muscle is allowed to thaw at room temperature it goes into a rigor which is characterized by spontaneous shortening resulting in a decrease in length of 70 per cent after 10 to 15 minutes and an obvious synaeresis producing a loss of weight of 35 per cent, changes which bear considerable resemblance to those occurring during the contraction of actomyosin threads. Although a load of 8 gm. is sufficient to prevent the shortening, when the load is removed the muscle will shorten to the normal extent even after it has been kept thawed and loaded for as long as 20 minutes.

The shortening takes place if muscle is frozen in the resting condition, after exhaustion by direct electrical stimulation, during isometric tetanus, and after it has been treated with inhibitors such as azide, cyanide, copper, hydrogen peroxide, p-chloromercuribenzoate, iodoacetate, and 2:4 dinitrophenol. It can be prevented, if before freezing, the iodoacetate poisoned muscle is exhausted, or left fixed at its resting length for several hours in N_2. After such treatment synaeresis is also absent on thawing. Shortening can be produced in these muscles by immersing them in a solution containing 0.0029 M ATP, 0.05 M KCl and 0.0005 M $MgCl_2$. Prolonged exposure to p-chloromercuribenzoate renders muscle inexcitable but the ATPase activity of myosin prepared from it and the shortening of thaw rigor are little affected. Similar although less clearcut results were obtained with hydrogen peroxide, indicating that in view of the effectiveness of these inhibitors on the enzyme activity of myosin *in vitro*, their action on the muscle cell must be confined to the membrane, or the myosin of the fibrils must be in some way protected from reacting with the inhibitors.

The phenomenon seems to be an in situ example of the synaeresis of actomyosin, but it does not necessarily follow that such system is responsible for muscle contraction. In fact there is a suggestion from this study that in muscle frozen while it is tetanised into maximal shortening, the actomyosin is still largely unsynaeresed, because synaeresis obviously takes place on thawing with a loss in weight only 27 per cent less than that shown by resting muscle.

KENNETH E. FISHER.

Considerable interest attaches to investigations dealing with the net movements of organisms to or away from some source of stimulation and to the mechanism of the aggregations of organisms in a particular region of a non-uniform environment. As an aid in such investigations, we have considered methods of describing the movements of organisms which would permit the construction of the hypothetical path traced out by an organism in a simplified situation. A rigid quantitative procedure has been devised by which this can be done. Using it the conditions required to predict the uniform distribution of organisms throughout a uniform environment have been determined as well as the nature of the "biases" necessary to bring about an aggregation of organisms in a non-uniform environment.

Time course studies of photosynthesis and respiration in unicellular algae using the platinum electrode with time selection. F. S. BRACKETT AND R. A. OLSON.

Observations of oxygen tension are obtained during respiration and photosynthesis in unicellular algae by means of a new method of oxygen determination employing an alternating potential and an accurate selection of time with the static platinum electrode. This method[1] provides accurate and reproducible determinations at 10-second intervals without the need of stirring or of moving components. By using small tubular cuvettes (2 mm. diam.) suspensions can be radiated from opposite sides by means of a dual monochromator system such that the intensity gradient due to absorption is minimal and all cells receive nearly equal radiation. This avoids the intermittent radiation of cells at widely varying intensities peculiar to the total absorption method in conventional manometry where unilateral radiation and stirring are employed. Typical time course records using this new method are presented and evaluated.

[1] Olson, Brackett, and Crickard, *Jour. of Gen. Phys.* (in press).

COMPARATIVE SEROLOGY OF SOME BRACHYURAN CRUSTACEA AND STUDIES IN HEMOCYANIN CORRESPONDENCE[1]

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Introduction

Researches in systematics may be conducted by comparing the serum or corresponding body proteins of organisms using serological methods. The underlying principle of serological systematics is that the proteins compared are representative of the organisms producing them in the same sense that their corresponding structures are and for the same general reasons. Of the serological reactions used for such researches the precipitin reaction has undoubted advantages. An extension of the previous precipitin studies in the serological systematics of Crustacea and related problems is the content of this report.

General Materials and Methods

Antigens

The sera of the Crustacea compared in this study were in part provided by Dr. Alan A. Boyden[1] who gathered them during summers over a period of years at various biological stations along the Eastern coast of the United States and elsewhere, namely, Mount Desert Island Biological Laboratory, Salisbury Cove, Maine, 1936; U. S. Bureau of Fisheries Laboratory, Beaufort, North Carolina, 1936; Tortugas Laboratory, Carnegie Institution, Washington; Key West, Florida, 1932, 1934, 1936, 1939; also at the Marine Laboratory, Citadel Hill, Plymouth, England, 1939, 1948.

In addition to the samples of sera, the author had access to Dr. Boyden's original records made at the time of collection, plus the correpondence incidental to the collection and identification of particular samples. These records were of great help in orientation to the work and in providing useful hints and suggestions which saved countless hours when the author did his own field collecting.

Useful serum samples were secured by the writer in the field at Barnegat Bay and Delaware Bay, New Jersey. Samples of European crustacean sera were collected in the Summer of 1948 by the author at the following European biological laboratories: Museum National D'Histoire Naturelle Laboratoire Maritime, Dinard, France; Universite de Paris, Biologie Marine, Laboratoire Arago, Banyuls-sur-Mer, France; and the Stazione Zoologica, Napoli, Italia.

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[1] This investigation was supported in part by a grant from the Research Council of Rutgers University. The author wishes also to express his sincerest thanks to Professor Alan A. Boyden, Director of the Serological Museum of Rutgers University, for his advice and encouragement, and for providing many of the serum samples used in this study.

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In general the blood was taken from the crabs by removing the 5th pereiopod and permitting the blood of the organisms to drain into porcelain pans or glass crystallizing dishes. The crabs were held over the collecting pans by means of an ordinary clamp attached to a cross bar supported between two vertical stands. In the case of the long-tailed Crustacea such as crayfish and lobsters, the blood was collected by slitting the abdomen ventrally where it joins the thorax and holding the organism by hand over a large collecting funnel making sure the posterior end of the abdomen was pressed firmly to the lip of the funnel as an insurance against sudden abdominal flexures. It is necessary to occasionally break the jelly-like clots that form at the openings from which the blood is draining in order to obtain good yields from each animal. In those cases where the organisms were small the blood was taken from convenient points within the body cavity by means of needle and syringe. Piercing the body at the base of the appendages usually gave good yields. It is interesting to note here that placing the needle in the heart or in the pericardium gave poorer results than when a sinus some distance from the heart was tapped.

TABLE I

List of the species of Crustacea used as antigens

Acanthocarpus alexandri Stimpson
Callinectes marginatus (Milne-Edwards)
Callinectes sapidus Rathbun
Cancer anthonyi Rathbun
Cancer borealis Stimpson
Cancer irroratus Say
Cancer magister Dana
Cancer pagurus L.
Carcinus maenas L.
Geryon quinquedens Smith
Maia squinado Rondelet (Herbst)
Menippe mercenaria (Say)
Mithrax verrucosus Milne-Edwards
Ocypode albicans Bosc.
Panopeus herbstii Milne-Edwards
Panulirus argus (Latreille)

The collected bloods were permitted to clot and the sera to be expressed. Centrifugation at 2000–3000 r.p.m. for 20 minutes was usually employed to clear the sera. It is feasible to hasten the expression of serum by wrapping the clot in a double thickness of clean, fine-mesh, bolting or cheese cloth and twisting the wrapping by hand. The sera were sterilized by Seitz filtration, bottled in serum vials and stored at 3° ± 1° C.

Table I is a list of the Crustacea whose sera were used as antigens in this work. The identifications of North American Crustacea were made with the works of Pratt (1936), Faxon (1898), and the remarkable publications of Rathbun (1917, 1925, 1930, 1937). The French fauna was checked against Bouvier (1940). The fauna of other European countries was identified in the works of Borradaile (1907), Pesta (1918), and Thiele (1935).

Antisera

All antisera were produced in rabbits. Intravenous and/or subcutaneous routes of injection were employed in the production of antisera. Inasmuch as it is impossible to ascertain at present the degree to which a rabbit will respond to a given amount of antigen, and since this response is perhaps the greatest variable in present day serological studies, what was believed to be minimal amounts to produce a good response were used. Generally 0.25 cc. was given on the first injection followed on alternate days with three 0.5 cc. injections. In every instance good, usable antisera were obtained when the hemocyanins were injected in these doses. In some cases a presensitization technique was employed to improve the strength (i.e. precipitating capacity) of the antisera. These rabbits were given 0.25 cc. of antigen intravenously and then permitted to rest for 30 days. This was followed by a series of 4 subcutaneous injections given on alternate days.

Table II

List of antisera

Antiserum	Homologous antigen	Remarks
I–63	*Menippe mercenaria* 36-A	Prepared by Dr. Alan A. Boyden Rutgers University
I–76	*Panulirus argus* 39–1	
I–77	*Panulirus argus* 39–1	
I–78	*Cancer borealis* 3a1	
I–86	*Callinectes sapidus* 47–1	
I–87	*Callinectes sapidus* 36–1	
I–94	*Cancer borealis* 1a	
I–95	*Maia squinado* No. 5	
I–97	*Cancer pagurus* No. 3	
I–98	*Menippe mercenaria* 36–A	
I–99	*Ocypode albicans*	
I–100	*Geryon quinquedens* 36–1	
I–101	*Cancer irroratus* 36–1	
I–105	*Geryon quinquedens* 36–1	
I–106	*Cancer irroratus* 36–1	
I–107	*Acanthocarpus alexandri*	
I–110	*Geryon quinquedens* 39–1	
I–114	*Callinectes sapidus* 47–1	

Table II is a list of antisera prepared and utilized by the author except where specially annotated.

Bleeding the rabbits to secure the antisera was accomplished in either one of two ways. Small samples of blood were withdrawn from the central artery of the ear, by using a number 22 gauge needle and syringe. For complete bleedings blood was withdrawn directly from the heart by cardiac puncture. Size number 18 gauge needles and 50 cc. syringes were used in this latter procedure. All antisera were centrifuged, sterile filtered through Seitz filters, and stored in the refrigerator until used.

Method of testing

The Libby photronreflectometer (1938) was utilized exclusively in the measurement of turbidities developed as a result of the interaction of antigens and antibodies. The technique employed was essentially the same as that described by Boyden and DeFalco (1943). Minor variations in technique as developed by the writer were matters of convenience and did not represent any major changes in their method of making the dilution series (see Figs. 1 and 2) of the antigens, nor in the use of the machine. Two recent papers (Boyden et al., 1947; Bolton et al., 1948) have analyses of the performance of the photronreflectometer and report the conclusion that for white precipitate systems, which include all the precipitin reactions, the instrument is unsurpassed at present in its sensitivity and range of usefulness in studying the characteristics of precipitates.

For all tests the procedure in which the amount of antiserum is held constant and the amount of antigen is varied was employed. The reacting cells of the photronreflectometer have a 2 cc. operating level and this volume was used in all testing. Final volume for each antigen dilution was always 1.7 cc. to which 0.3 cc. of immune serum was added to make up the 2 cc. volume. Turbidities (i.e. galvanometer readings) inherent in the fluid of the antigen dilution, and those due to dirt or blemishes on the glass of the reacting cells plus the turbidity characteristic for 0.3 cc. of each antiserum used were deducted from the total turbidity developed in each reacting cell. For these reasons the resultant turbidities can be considered as those due to the interaction of antigen and antibody.

The range of antigen dilutions regularly employed was between 4000 and 1 gamma of protein per cubic centimeter or solution, or in terms of dilutions of protein from 1:250 in doubling series to 1:1,024,000. When necessary the range of antigen dilution was extended. For the sake of convenience in plotting the results of the reaction on graphs, the antigen dilution cells were assigned numbers in a chronological sequence with cell No. 1 containing the greatest concentration of antigen. Figure 1 shows graphically a typical dilution sequence.

For all of the work in this paper the precipitin turbidities were those developed during 20 minutes incubation in a dry-air incubator maintained at 38° C.

The titration curve

The graph of the turbidities developed for serological tests is usually made in this laboratory with the turbidities plotted as the ordinate and the antigen dilution as the abscissa with the greatest concentration of antigen nearest the x-y axes intersect. The turbidities rise to a maximum, then decline again to a minimum following generally a normal distribution curve. By assigning unit distances between the geometrically changing antigen concentration values along the abscissa, the curves as plotted are more or less symmetrical. Skewness toward the region of antigen excess is quite common. Variations in the amount of kurtosis have also been observed. The amounts and kinds of both antigens and antibodies in the solutions combine to provide many variations of this type of frequency distribution. Figure 2 represents an idealized titration curve. The numerical value used to characterize any one particular curve for comparative purposes is that obtained by summating the turbidities over the whole reaction range. This value is proportional

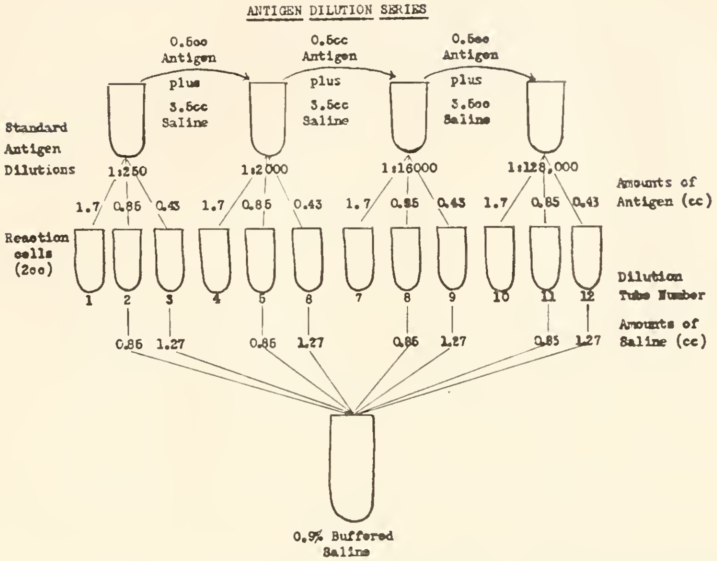
ANTIGEN DILUTION SERIES
0.5cc Antigen plus 3.5cc Saline
0.5cc Antigen plus 3.5cc Saline
0.5cc Antigen plus 3.5cc Saline
Standard Antigen Dilutions
1:250
1:2000
1:16000
1:128,000
1.7 0.86 0.43
1.7 0.86 0.43
1.7 0.85 0.43
1.7 0.85 0.43
Amounts of Antigen (cc)
Reaction cells (2cc)
1 2 3 4 5 6 7 8 9 10 11 12
Dilution Tube Number
0.86 1.27
0.86 1.27
0.86 1.27
0.85 1.27
Amounts of Saline (cc)
0.9% Buffered Saline

FIGURE. 1. A diagram of the technique used in preparing a typical doubling-dilution series of a given antigen for use in the Libby Photronreflectometer. The initial standard antigen dilution (1:250) is prepared directly from a serum of known protein concentration. The subsequent standard antigen dilution tubes are prepared from the first. Each reaction cell has a constant antigen dilution volume of 1.7 cc. to which is added and mixed a constant volume of 0.30 cc. of antiserum (α procedure).

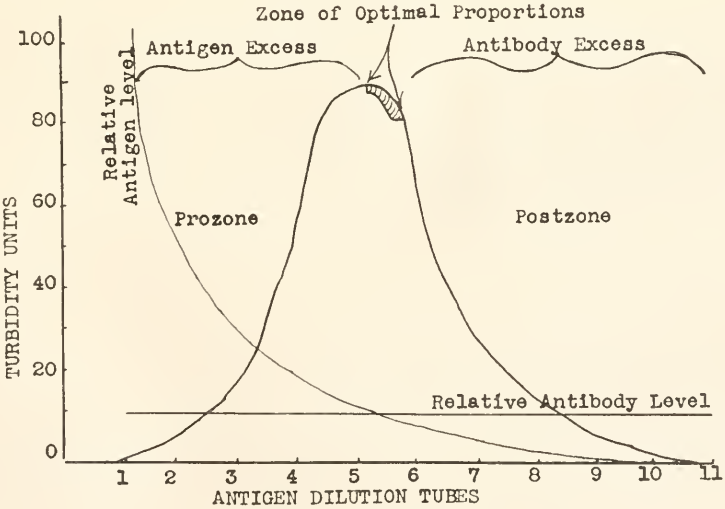

FIGURE 2. A diagrammatic representation of a typical titration curve obtained by using the Libby Photronreflectometer as the turbidimeter, and the technique of reacting varying antigen dilutions with a constant amount of antibody. Included also are appropriate curves to portray the relative antigen and antibody levels in each antigen dilution tube. For these latter curves the turbidity units of the ordinate axis do not apply.

to the area under the curve and provides an easily obtained statistical index of a very complicated biochemical system.

For all titrations the dilution medium was 0.9 per cent NaCl buffered with M/15 phosphate salts (Sorensen's solution) (Evans, 1922) such that the buffer was in a final concentration of M/150. The pH range for the tests was between 7.05 and 7.15.

Antigen Correspondence

The continuous analysis of the properties of both of the primary reagents used in serological work is necessary if the investigations are to be considered critical. This problem is especially pertinent to workers in the field of serological systematics since they must be certain that the sera or proteins or organisms used in their tests are unchanged from the native state, or if changes have occurred because of prolonged storage or other physical or chemical factors, they must be prepared to correct for it.

The sera of Crustacea contain one principal protein, hemocyanin (Allison and Cole, 1940). They are excellent antigens when injected into the rabbit. They are usually considered to be relatively pure systems. However, electrophoretic patterns (Cohn and Edsall, 1943), sedimentation constants (Dawson and Mallette, 1945; Redfield, 1934), and $(NH4)_2SO_4$ precipitation (Bolton, personal communication) all indicate that this single serum protein may be composed of several molecular "species" of hemocyanin. The hemocyanin molecules are large with molecular weight ranging from 300,000 upward to several millions as calculated by Svedberg and his collaborators from data obtained by using the ultracentrifuge methods of sedimentation velocity and sedimentation equilibrium. A glance at almost any titration curve of the hemocyanin antigen-antibody system (Fig. 3) will show irregularities and disturbances in the modality of the plot. Occasionally a titration will show two distinct modes. These variations from a single mode frequency curve have long been considered as evidence of the probable presence of more than a single kind of antigen or antibody in the system. It is quite likely that the above mentioned molecular "species" are responsible for the stimulation of more than a single principal kind of antibody in the rabbit serum. This, of course, does not preclude the possibility that a single molecular "species" could stimulate the production of two or more distinct kinds of antibodies. Until such time as this can be proven, it is simpler to assume that a single kind of antibody is produced against each kind of antigen, and that the appearance of additional modes in the titration curve of any antiserum is due to the reaction of individual molecular "species" in the antigen complex with their homologous antibodies. This assumption is not inconsistent with the observed behavior of antigen-antibody systems.

The serological identification of all proteins resides in their structural peculiarities and in their chemical nature, i.e., the kinds, proportions and arrangement of the amino acids and prosthetic groups all are believed to affect the serological activity of proteins. It is well known that mild treatment both chemical and physical, will alter the nature of proteins (Landsteiner, 1936; Cohn and Edsall, 1943). This fact presents a challenge to all investigators who are using animal proteins as representing the nature of the organisms with which he is working. Studies in serological systematics may require that animal sera be collected and saved, sometimes for a period of years, before numbers of different species sufficient for comparative in-

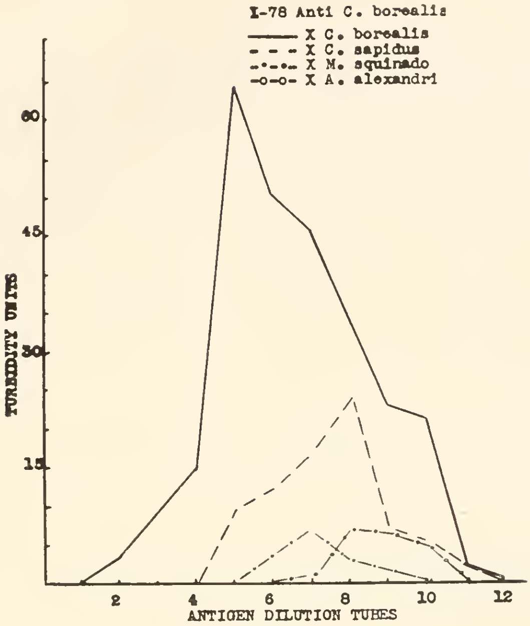

FIGURE 3. A typical series of precipitin titration curves showing an order of serological relationship among four crabs. The homologous reaction between *Cancer borealis* and the antiserum produced against it exceeds all others. Then the alignment occurs, in the order of their decreasing curve areas, *Callinectes sapidus, Acanthocarpus alexandri,* and *Maia squinado,* which is in accord with accepted systematic relationships.

vestigations are secured. Further, sera frequently have to be collected under field conditions, necessitating the use of preservatives. There are other aspects of the general problem of antigen comparability than those resulting from the chemical action of preservatives which must also be considered before comparisons of animal sera can be made with confidence. Following are the results obtained from attempts to test the affect of some of the variables encountered in the preparation of animal sera for comparative studies in serological systematics.

Physical treatment

Usual laboratory procedures have been to allow the blood to clot and the sera to be expressed for about 24 hours. The sera are then centrifuged at 2000–3000 r.p.m. for 20 minutes, filtered sterile in Seitz filters, bottled, and stored in the refrigerator at 3° ± 1° C. Any or all of these steps could alter the nature of the serum proteins and thus modify their serological activity. Table III summarizes a series of tests performed to examine the effects of treatment in the laboratory. The sample of blue crab serum designated in the table as *Callinectes sapidus* 47–2 represents the sera of 24 large male crabs all bled within one hour. The pooled collection of sera was divided into several parts and given various kinds of physical treatment in the laboratory. All of the antigens were tested against antiserum I–86 (Anti-*C. sapidus* 47–1) which was prepared against a fresh, sterile filtered sample of crab serum. The antiserum I–86 was powerful and was diluted with 10 parts buffered saline at pH 7.0. Since dilution is known to increase the specificity of an antiserum (Boyden and DeFalco, 1943) under certain conditions it was advantageous to dilute the antiserum in this manner to magnify any differences among the antigens. The dilution factor was so chosen that total turbidities would summate in the vicinity of 300 galvanometer units. A curve area this large tends to minimize variations in results due to errors in experimental techniques. Experimental error is limited to 5 per cent. In addition to the treatments listed in the table attempts were made to lyophilize (freeze-dry) (Florsdorf and Mudd, 1935) parts of this sample, but "boiling"[3] occurred during the course of drying, which denatured the proteins to such an extent that insufficient amounts of them could be restored to conduct comparative studies.

TABLE III

Effect of laboratory handling on antigen reactivity

Antiserum I–86 Anti *C. sapidus* 47–1 (1 + 10) × *C. sapidus* 47–2

Treatment	Area	% Change
Centrifuged, filtered	279	0
Centrifuged, unfiltered	283	1
Uncentrifuged, unfiltered	287	3
Frozen, centrifuged	287	3
Frozen, uncentrifuged	290	4
Room temperature, centrifuged, filtered	275	1
Room temperature, centrifuged, merthiolate	285	2

The homologous antigen *Callinectes sapidus* 47–2 was centrifuged and sterile filtered before it was used as an antigen. Changes due to above listed treatments appear to be negligible.

It is realized that any tests involving comparisons with only a single antiserum prepared from but one kind of the possible antigen types present but a minimum of data on the comparability of antigens. Moreover, in the type chosen (Table III, explanation), it still is possible that the unfiltered, and the frozen samples could have constituents not possessed by the filtered samples. That there was a high degree of correspondence among all of the antigens tested is testimony to the fact that the unfiltered, and frozen samples contain corresponding antigens to those in the filtered material, also that filtration does not significantly reduce the quantity of such antigens nor the quality of their reacting (combining) capacity. For the short period of time (24 hours) involved between the beginning and end of the processing, the sample held at room temperature showed no difference from the others. A retesting of the room temperature sample seven days later also revealed no significant change in activity.

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[3] This is the bubbling which occurs when the rate of sublimation of the frozen material undergoing desiccation is slow and thawing occurs at the inside glass surface of the containers because of the transfer of atmospheric heat to the frozen solid while the whole system is under vacuum. The relationships between the rate of heat intake from the atmosphere at the exterior glass surface of the containers, the rate of heat loss at the evaporating surfaces of the product and the rate of escape of water vapor from the product to the condenser is apparently upset.

Effect of cold storage

The question of antigen comparability is bound to the problems related to the storage of sera for prolonged periods. In order to examine adequately the effects of storage it was necessary to establish whether or not significant alterations occurred in samples of sera of various ages. Table IV summarizes the results of these tests. It is readily seen that no demonstrable immunological alteration occurred between the samples one day old (22 hours) and the other older samples.

TABLE IV

Antigen comparability

Sample	Number of individuals	Per cent protein	Whole curve area	Per cent deviation	Age of sample when tested
47–3	12	7.04	280.1	−0.3	22 hours
47–2	12	7.00	278.8	−0.1	1 month
*47–1	24	7.39	279.1	0.0	4 months
36–1	5	5.05	281.8	−0.9	11 years

Table showing the lack of change in serum samples of the blue crab, *Callinectes sapidus*, when tested with an antiserum against one of them (*). Samples stored at 3° ± 1° C.

The fact that the eleven year old sample number 36–1 of *C. sapidus* possessed a precipitinogen activity equal to the very fresh sera of this species is very surprising and important. This old serum has been kept at refrigerator temperatures practically continuously since it was collected in 1936. The amount of alteration that goes on in these serum proteins, if any occurs, must be very slight not to be detected by immunological testing. All the samples listed in Table IV represent pooled samples of the sera of five or more animals. It is conceivable, however, that individual variability of the sera does exist and that the pooling nullifies this variation, presenting for test a more or less "common denominator" kind of serum to the testing antiserum.

Serological variation

Interesting evidence of the stability and lack of variability among various samples of sera of the same species of spiny lobsters, *Panulirus argus*, is illustrated in Table V. Here samples collected over a period of years (1932–1939) were tested against an antiserum made to one of them. The test antigens were selected to reveal the differences due to aging, differences due to sex, and differences between single specimen samples and pooled samples containing the sera of more than one individual. The amount of difference in the serological reactivity among these different categories was negligible. In no instance did the variations in the whole curve areas exceed the experimental error of the method of testing. This is a remarkable fact, considering the length of time these sera have been stored; the youngest sample being nine years old at the time of testing, the oldest sample being fifteen years old.

Considering the sensitivity of the precipitin tests to slight alterations in chemical structure, the lack of variation in the amount and kind of reactivity demonstrated in the data of Tables IV and V is testimony first to the care in the laboratory prepa-

TABLE V

Antigen comparability

Sample	Number of individuals	Per cent protein	Whole curve area	Per cent deviation
32–1	"Pooled"	6.07	345.3	−0.2
32–2	"Pooled"	6.52	345.6	−0.1
34–C	1	4.55	351.6	1.5
34–D	1	5.33	340.8	−1.4
34–E	1	2.66	357.0	2.9
34–F	1	7.66	355.7	2.8
34–G	1	3.69	343.7	−0.6
36–1	1	5.31	345.2	−0.2
36–3 & 4	2	10.05	353.6	2.5
36–5 & 6	2	7.44	350.6	1.3
36–10	1	6.07	354.5	2.5
36–11	4	6.52	351.3	1.5
*39–1	5	4.59	345.9	0.0
39–3	9	6.02	349.4	0.2

Table showing the high degree of serological similarity among different samples of the spiny lobster, *Panulirus argus* collected between 1932 and 1939, and tested with an antiserum against one of them (*). Tests were conducted in 1947.

ration of the sera, and the use of a correct method for maintaining useful samples for long periods of time, and second, a testimony to the durability and stability of hemocyanin proteins when collected and preserved in the manner described above.

Color changes

Hemocyanin proteins possess a copper radical and in the oxidized state and in vitro have a blue to green color when in solution. Occasionally, variations have been noted in the color of the sera after they have been sterile filtered and bottled. The most frequent variation is a change from the blue-green color to dark brown. This change is due in part to the free carbon on the Seitz pads which forms there as a result of over-heating during sterilization of the filter, and also to chemical changes in the proteins themselves since the color change has been noted not only during the filtration processing but also in some samples previous to filtering. The brownish color does not appear to play any role in altering the reactivity of the sera since these samples compare favorably with others of the same species possessing the blue-green color.

If a sample of hemocyanin serum is contaminated with bacteria, these organisms flourish and the blue-green, oxidized condition of the serum changes to a colorless liquid, with a cloudy suspension of bacteria. If such sera are kept in the refrigerator, even though contaminated, no recognizable alteration occurs in the activity of the dissolved protein when tested against an antiserum. Removal of the bacteria by filtration or centrifugation or both quickly restores the oxidized condition of the serum. Its serological behavior seems not to be altered even when the serum has been stored in the contaminated state in the refrigerator for prolonged periods.

Precipitates in vials

Another phenomenon that has been seen to occur in sterile, bottled sera is the appearance of precipitates in the vials. In some instances the amount of material coming out of solution has been considerable. The supernatants of many of these vials were examined and found still to contain sufficient protein in solution to warrant testing for comparability with nonprecipitated samples of the same species. Surprisingly enough the still soluble fractions of the total proteins in the antigens apparently possessed all of the reactivity characteristic for the serum. This would indicate that the precipitate is denatured protein which has not undergone any appreciable decomposition. The presence of large amounts of free amino acids and peptides in solution above the precipitates would present free radicals that might combine with the antiserum, blocking the reaction and causing some difference in the amount of reaction observed to occur between the still soluble protein and the antiserum. This is not the case with these systems. The precipitates, moreover, still possess the capacity to combine with antisera, as saline suspensions of them readily reveal. The antigen precipitates go back into solution readily using dilute alkali, but not in saline solutions up to 1.7 per cent NaCl.

Serological Systematics of Some Crustacea

Using the procedures described above, it thus appears feasible to collect and store the sera of Crustacea for long periods of time (15 years in the data given) without significant alteration in their specific properties. Comparative studies of the various species of Crustacea become in effect analysis of the biochemical nature of the organisms concerned based on the quantitative comparison of the nature of their serum proteins, as reflected by turbidities developed in the antigen-antibody reaction.

Erhardt (1929) reviewed the early work done on serological systematics among the Crustacea. Some of these early investigators obtained results which do not agree with systematic classifications based on morphological and embryological data. In one instance Nuttall and Graham-Smith, each using the same antiserum, compared exactly the same organisms and obtained very different results. In most other instances the serological work generally agreed with well established classifications. Most all of these early tests were done using the interfacial or "ring" test method for comparisons. The correspondence of techniques among the various workers ended there because the antigens were not standardized and because results of tests based on antiserum titers and those based on antigen titers cannot be directly compared.

Boyden (1942) pointed out the deficiencies and inadequacies in these early serological investigations and in two papers (1939, 1943) presented the first really quantitative study in the serological systematics of the Crustacea. He compared representatives of five families of the Brachyura and two species of the Macrura. It was in the later paper that the concept of the "serological yardstick" was introduced. The relationships of the sera of species of the same genus, and genera of the same family, and different families of the tribe Brachyura were remarkably consistent in their serological values. All of his tests were made using the photronreflectometer and standard procedures as outlined by Boyden and De-

Falco (1943). The inconsistencies apparent in earlier investigations by other workers were not observed to occur in these studies.

The availability of a larger and more representative group of Crustacea made an extension of Boyden's work possible. Table VI summarizes the serological comparisons made. It should be pointed out that the values given are averaged from two or more tests. The generally higher interspecific, intergeneric and interfamily relationships than those reported by Boyden seem to be due to the use of the presensitization technique to produce more powerful and less discriminating antisera.

From Table VI it is apparent that the antisera differ in their capacities to discriminate among the families of Brachyura. It appears that the Portunidae, Xanthidae and Cancridae are more closely related to each other than they are to the Ocypodidae, Calappidae and the Majidae. Of special interest is the species *Geryon quinquedens* here listed as a member of the family Goneplacidae, in accordance with Rathbun's classification (1937). She concluded that Goneplacidae were closely related to the Xanthidae. Bouvier (1940) places Geryon in the family Xanthidae. The serological tests indicate a degree of correspondence for *Geryon* which is approximately the same in heterologous reactions as for the xanthid species. Further critical testing is necessary to definitely establish the affinities of *Geryon*.

TABLE VI

The relative relationship among Crustacean species representing seven families in the tribe Brachyura

Antisera	Antigens (Relationship in per cent)												
	Callinectes sapidus (Portunidae)	*Callinectes marginatus* (Portunidae)	*Carcinus maenas* (Portunidae)	*Cancer borealis* (Cancridae)	*Cancer irroratus* (Cancridae)	*Cancer pagurus* (Cancridae)	*Cancer anthonyi* (Cancridae)	*Panopeus herbstii* (Xanthidae)	*Menippe mercenaria* (Xanthidae)	*Ocypode albicans* (Ocypodidae)	*Acanthocarpus alexandri* (Calappidae)	*Geryon quinquedens* (Goneplacidae)	*Maia squinado* (Majidae)
Callinectes sapidus (Portunidae)	100	78	46	33	44	37	33	24	48	18	12	38	8
Cancer borealis (Cancridae)	29	22		100	75	54		20	42	9	8	22	9
Cancer irroratus (Cancridae)	49	46	33	78	100	73	72	30	35	43	10	31	30
Menippe mercenaria (Xanthidae)	27		21	24	21	16	24	37	100	8		27	3
Ocypode albicans (Ocypodidae)	30		40	33		34		39	34	100	39	6	33
Acanthocarpus alexandri (Calappidae)	12		13	15		15				18	100	14	2
Geryon quinquedens (Goneplacidae)	15			13					15		8	100	9
Maia squinado (Majidae)	21			16					13	13	8		100

From Table VI it is readily seen also that species within a given genus react with each other to a greater extent than with any other organisms.

The problem of establishing in detail interfamily relationships among Crustacea by serological methods is one that would entail the production of large numbers of antisera in an attempt to secure serological reagents which are sufficiently powerful to react significantly with the more distant families and at the same time discriminate among the representatives of these families sufficiently to establish a verifiable order of relationship.

SUMMARY

Serological Systematics is a branch of Serology concerned primarily with the classification of organisms. The taxonomic characters usually concerned are the serum proteins of organisms. The natural relationships obtained are those revealed by an antiserum in combination with its homologous antigen and various heterologous antigens which react in proportion to their degrees of correspondence to the homologous antigen. Many factors influence the antigen-antibody reaction. A few conditions which are of importance to studies in systematic serology have been investigated and the results given.

Antigens tested for comparability under a variety of the circumstances met in ordinary laboratory handling such as freezing, filtration and centrifugation, showed no significant deviation from each other in their serological activity. Age, within the limits stated, was shown to have no effect on the serological activity of serum antigens which are sterile filtered and stored just above freezing. Antigens in cold storage for as long as 15 years had the same activity as freshly prepared samples. Pooled serum antigens showed no serological differences from the sera of individuals. No differences were demonstrated in sera due the sex of the organisms. For cold stored antigens color changes in the vials did not indicate alteration of the reactivity of the proteins. The remaining soluble portions of protein in vials showing apparently spontaneous precipitation of the protein gave the same reactivity as freshly prepared antigens. Bacterial contamination, if not permitted to endure too long, and if kept under refrigeration, does not alter the proteins significantly. The reconstitution of lyophilized hemocyanin sera for serological testing was not successful.

The studies in systematic serology have been extended to include new families of decapod Crustacea. For the species of Brachyura tested it appears that the families Portunidae, Xanthidae, and Cancridae are more closely related to each other than all of them are to the Ocypodidae, Calappidae and Majidae.

Further studies into the conditions which may modify the serological reactivity of proteins are needed. Continuing investigations, examining both the validity of the methods used in measuring antigen-antibody reactions and the methods used in the preparation of these primary serological reagents, are necessary components of truly critical studies in serological systematics.

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THE GROWTH AND METAMORPHOSIS OF THE ARBACIA PUNCTULATA PLUTEUS, AND LATE DEVELOPMENT OF THE WHITE HALVES OF CENTRIFUGED EGGS

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The pluteus of *Arbacia punctulata* which is well known to many investigators is the early pluteus with four arms, a pair of long anal (post-oral) arms on the ventral side, and a pair of short oral (pre-oral) arms on the dorsal side. In order to obtain further development of the pluteus, it is necessary, at Woods Hole, to feed the animals a rather special diet. In 1882 W. K. Brooks and two of his students, Garman and Colton, raised the plutei of *Arbacia punctulata* at Beaufort, N. Carolina, apparently without any special feeding. The sea water there is rich in diatoms, and the plutei can probably obtain what they require for growth from the sea water. This work was published by Brooks (1882) in his Handbook of Invertebrate Zoology, and by Garman and Colton (1883). The drawings are excellent for detail, but there is no indication of size and not adequate indication of age. A few stages had previously been described and figured by Fewkes (1881) working in A. Agassiz's laboratory at Newport, R. I. The present account with photographs gives the development of the pluteus with regard to size, sequence of events and rate of development at Woods Hole.

The best food for sea urchin larvae has been found to be the diatom, *Nitzschia closterium;* I found that they would also grow on the diatom, Licmophora. The Nitzschias themselves require a special diet, and must be raised in pure culture; they are raised on Miquel's solution.[1] The method has been worked out by Allen and Nelson (1910) in Plymouth, England, and has been used by many investigators at the Plymouth laboratory. Shearer, deMorgan and Fuchs (1914) have in this way succeeded not only in raising the normal plutei of several species of sea urchin to maturity, but have also raised some hybrid plutei to maturity. Fuchs (1914) has even obtained the next or F_2 generation of these hybrids. Unfortunately, the late

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[1] Miquel's solution, as modified by Allen and Nelson (1910), consists of:

Solution A	KNO_3	20.2 grams
	Distilled water	100 cc.
Solution B	$Na_2HPO_4 \cdot 12\ H_2O$	4 grams
	$CaCl_2 \cdot 6\ H_2O$	4 grams
	$FeCl_3$ (melted)	2 cc.
	HCl (concentrated)	2 cc.
	Distilled water	80 cc.

To each liter of sea water add 2 cc. Solution A and 1 cc. Solution B, and sterilize by heating to 70° C. When cool, decant off the clear liquid from the precipitate, which will have formed when Solution B is added to the sea water.

Ketchum and Redfield (1938) have used a slight modification.

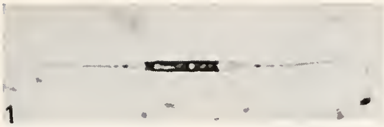
larval characters of *Echinus esculentus* × *E. acutus* from which the F_2 generation was obtained are alike in the two species, so that no information as to inheritance could be obtained, and none of the F_2 hybrids between *E. esculentus* or *E. acutus* × *E. miliaris* which would have given the information, reached maturity. Miss Gordon from MacBride's laboratory raised some Arbacia plutei at Woods Hole in 1926, using this method, but she was particularly interested in the later development of the test, and gives no account of the changes in the pluteus in her publication (1929).

There are two forms of *Nitzschia closterium* both of which are devoured by the plutei. One, the large form (Plate I, Photograph 1) is about 100 μ long; the other, forma *minutissima* (Photograph 2), is about 24 μ long and is the variety used in the Plymouth laboratory. The Nitzschias are swept into the oesophagus and stomach (Photographs 3, 4) by cilia.

Several stages in the early development from the fertilized egg are shown on Plate II (Photographs 1–6). The micromere stage (Photograph 2) is the first sign of differentiation of cells, the micromeres being small and colorless. It also marks the beginning of asynchronous cleavage; in Arbacia there is a definite 12-cell stage preceding the 16-cell stage. With further cleavages a blastula is formed and emerges from the fertilization membrane (Photograph 3) in about 8 hours, the time varying by one or two hours in different batches and with different temperatures. At this stage I have estimated that there are 1,000 to 2,000 cells representing 10 to 11 cleavages (2^{10} to 2^{11}). The blastocoel becomes larger, leaving a single layer of peripheral, ciliated cells (Photograph 4). Then invagination takes place (Photograph 5), and a gastrula is formed (Photograph 6). At this time the skeleton appears in the form of triradiate spicules, one on each side of the gut. During this period there is no appreciable increase in size of the organism over that of the egg (without the fertilization membrane), and one would not expect an increase before the alimentary canal is complete and it can take in food from the outside. Now growth occurs and differentiation into the pluteus form with skeletal rods on each side (Photograph 7). At this stage the large pigment spots characteristic of the later plutei begin to form. The young pluteus increases in size and the arms begin to grow out (Photograph 8). The pluteus is well formed in 24 hours (Photograph 9). It is larger on the second day (Photograph 10), usually reaching a maximum in three or four days (Photograph 11). The long anal arms may measure 410 μ from base to tip. Without special feeding, the pluteus may live three or four weeks, gradually getting smaller by resorption of its arms (Photographs 12–14). It has apparently obtained sufficient food for growth from the sea water for the first four days, but then requires additional food. The structure of a two day pluteus is shown in serial photographs (Plate I, Photographs 5, 6, 7). These are taken at different levels through the animal, corresponding to serial sections of imbedded material.

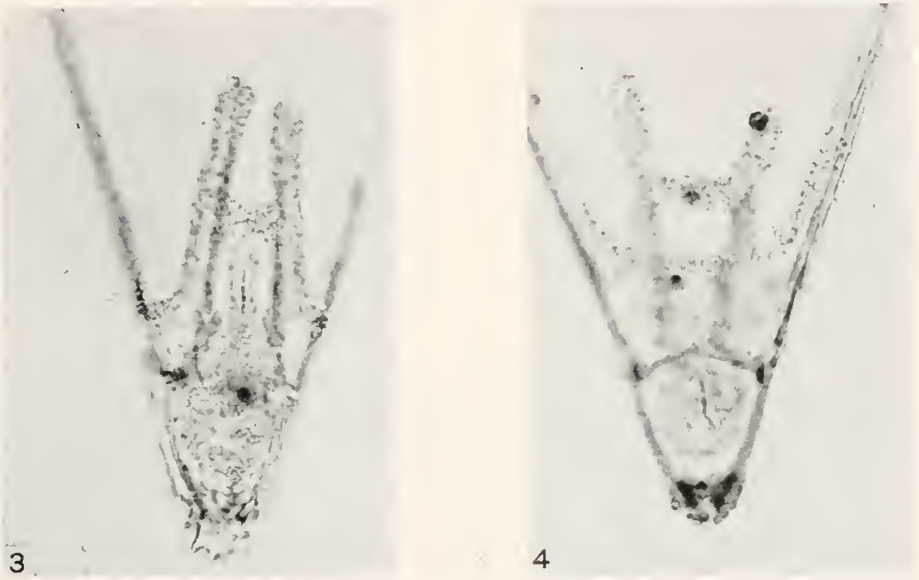
Further development of the three or four day pluteus may take place if *Nitzschia closterium* is added to the cultures of plutei. Only a few plutei in any culture continue to develop. The British investigators have found it expedient to have only a few individuals in a large amount of water, 20 to 30 in a half-gallon jar. (See MacBride 1914, p. 506.) I raised them in Syracuse watch glasses holding about 15 cc. of sea water with about a dozen plutei in each dish. The developing larvae

Plate I

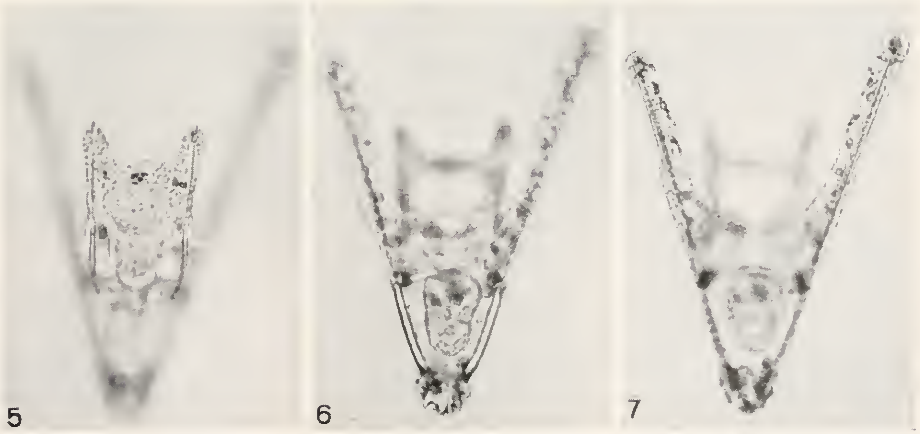


Nitzschia closterium

Nitzschia closterium
forma minutissima



Plutei engulfing Nitzschias

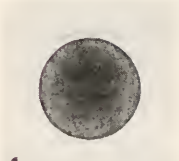


Serial photographs of 2 day pluteus

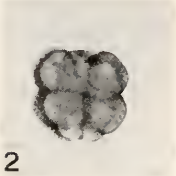
Dorsal Median Ventral

Plate II

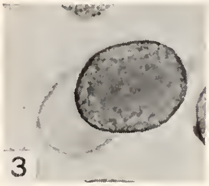
DEVELOPMENT WHEN NOT FED


1

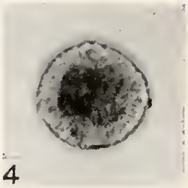
Fertilized egg


2

2½ hrs.


3

8 hrs.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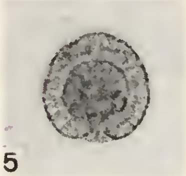
12 hrs.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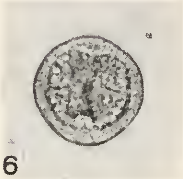
15 hrs.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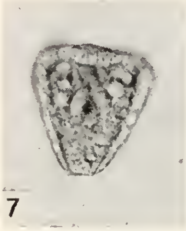
17 hrs.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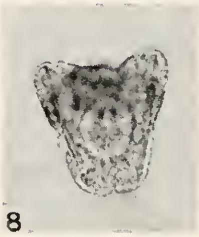
19 hrs.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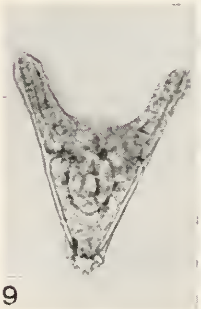
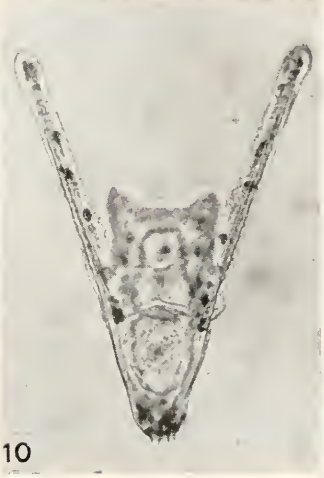
21 hrs.


9

1 day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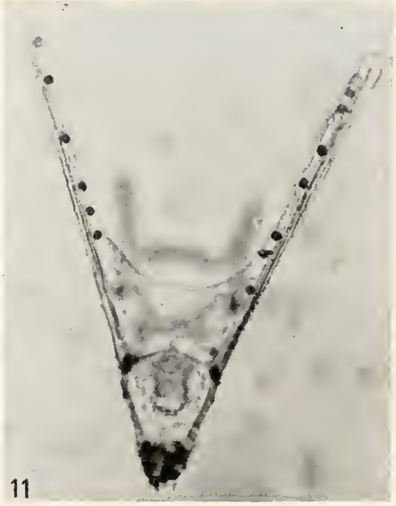
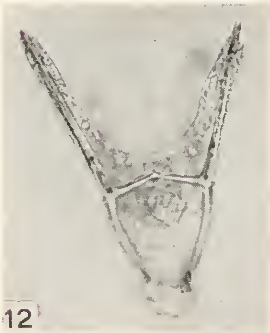
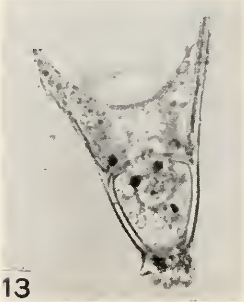
2 days


11

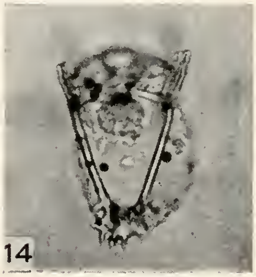
4 days


12

6 days


13

9 days


14

13 days

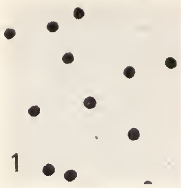
were transferred to fresh sea water with a pipetteful of the Nitzschia culture every few days. The eggs from which the final small adults were obtained were fertilized on July 12th, 1948, at Woods Hole, and taken to Princeton, N. J., on October 3rd. The last four died after completing metamorphosis on November 17th, a little over four months old.

The photographs on Plates III and IV, 1–18 are all to the same scale. The development varies in time in different batches, and in individuals of the same batch, so that the times given are only approximate. The eggs at this magnification (approximately 24 times) are of the size shown in Photograph 1. The one and the three day plutei to this scale are shown in Photographs 2, 3, shown with a larger magnification in Photographs 9, 10, 11 on Plate II (approximately 180 times). For the first four days, the development is the same whether fed Nitzschia or not, the food in the sea water being adequate. When fed, in about a week (Photograph 4, Plate III), the anal arms have become considerably longer. Then little knobs appear toward the base of the animal, which, by the eleventh day have grown out into a definite pair of new arms, the ventral lateral or postero-lateral (Photograph 5). These are always heavily pigmented at the tips which appear very red. These grow longer and a pair of knobs appear between the original anal arms and the new red-tipped arms. These are slightly noticeable in Photograph 6. In a month's time, these have grown into a second new pair of arms (Photograph 7); these are the dorsal lateral or postero-dorsal arms. These arms usually do not have red tips, although sometimes all the arms are pigmented at the tips. All the arms become continually longer (Photograph 8). The animals are now easily visible to the naked eye and look like small spiders. The arms are variable in length individually and relatively to each other. They are all ciliated, the cilia on the red-tipped ones being much longer and stronger than those on the other arms. The animal now swims actively by means of its cilia, and also walks or tumbles about on the tips of its arms, which can be readily moved. The arms are quite fragile and are easily broken off when the animal bumps into something or when it is transferred to another dish. They have great regenerative capacity, the arms growing out again when broken off. One pluteus, from which I had cut off the red-tipped arm about half way down, had completely regenerated it together with the red pigment in five days so that it then looked exactly like its mate. The broken piece may seal itself off and swim about actively by means of its cilia like a complete organism, looking something like a paramoecium (Plate VI, Photograph 6). One of these pieces was alive and active for four days when it was inadvertently lost. The body of the adult Arbacia is seen as a yellowish green mass in the pluteus, the dark area in Photographs 7, 8 (Plate III) and thereafter. There are areas of dark red pigment on the surface. The young adult is formed in the body of the pluteus and grows at the expense of the pluteus.

By six weeks the pluteus has become quite complicated, with two pairs of secondary oral arms, and four tubular processes (auricular lobes), two dorsal and two ventral. In Photograph 9, one sees several of the oral arms (at the anterior end) and a pair of the tubular processes (at the posterior end). A diagram of this stage is given by Miss Gordon (1929, p. 291). Soon after this, the five primitive ambulacral feet appear at one side of the body; these have suckers at their extremities and are continually expanded and contracted (Photograph 10).

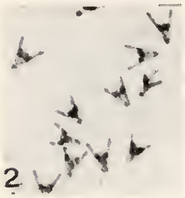
PLATE III

DEVELOPMENT WHEN FED



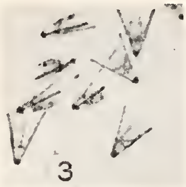
1

Eggs to scale



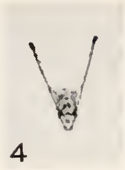
2

1 day



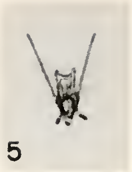
3

3 days



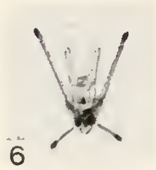
4

1 week



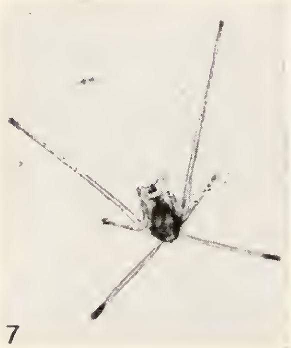
5

11 days



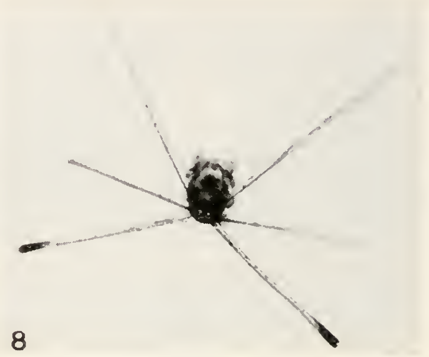
6

3 weeks



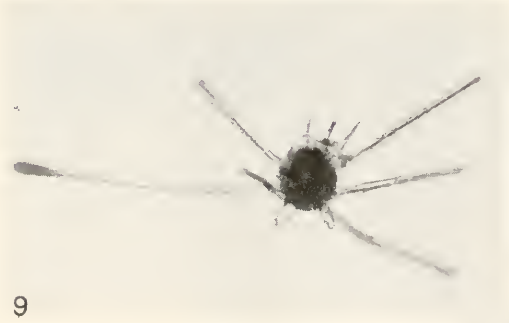
7

1 month



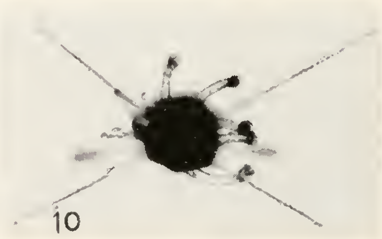
8

5 weeks



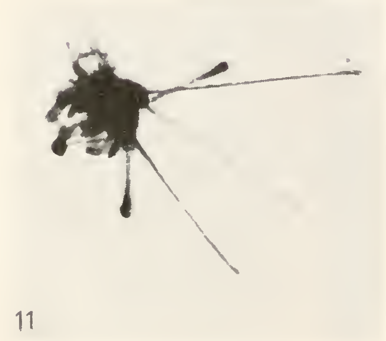
9

6 weeks



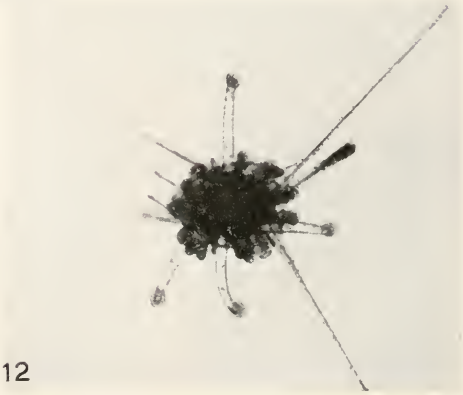
10

2 months



11

2 months

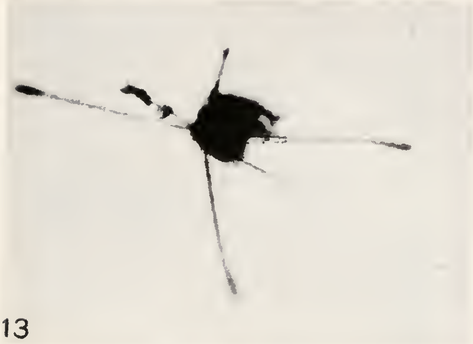


12

2½ months

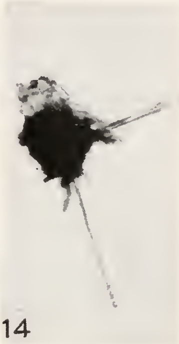
Plate IV

DEVELOPMENT WHEN FED II



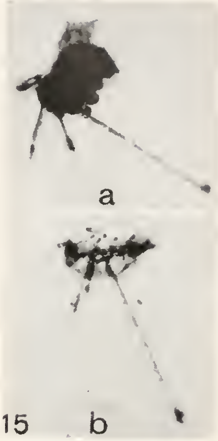
13

3 months



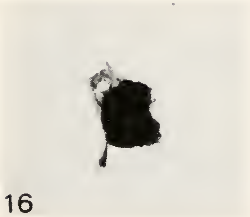
14

4 months



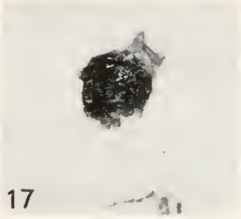
15

4 months



16

4 months



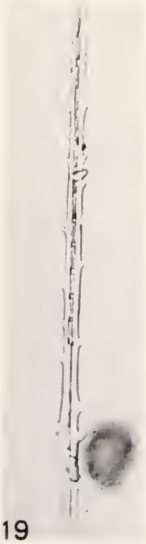
17

4 months

18

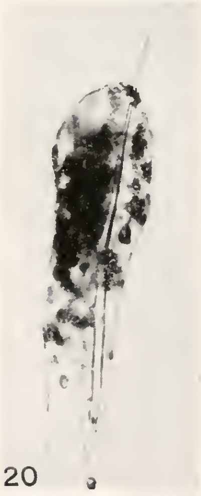
4 months

ARM SKELETON



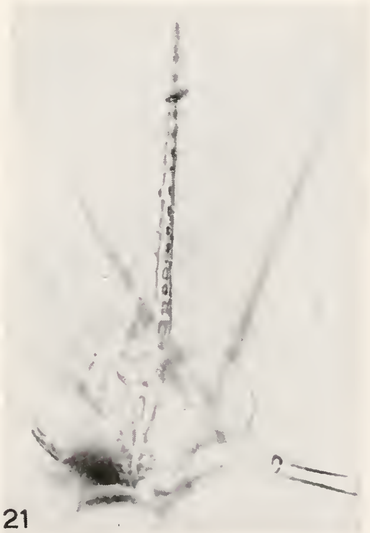
19

Original



20

First new



21

Second new (middle)

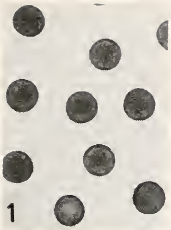
In Photograph 11, also a two months pluteus, one sees the three pairs of long arms, two pairs of the short arms near the head of the pluteus which is still prominent, the five ambulacral feet, with suckers, and the body of the developing adult. After two or more weeks, between each two ambulacral feet are formed three flattened plates, the first set of spines (Photograph 12). The pluteus has now reached its full development and the arms their maximal length, ca. 1.6 mm. The length of the arms is quite variable in different individuals even of the same batch. To give some idea of the increase in length of the arms, the following table is given of the length of the long anal arm at different ages. The figures represent measurements of the average better developed ones, the poorly developed ones not being taken into account.

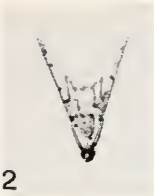
Approximate length of long (anal) arm from base to tip (μ)

	Fed	Not fed
1 day	180	180
2 day	300	300
3 day	380	380
4 day	410	410
5 day	450	330
6 day	480	250
1 week	600	200
11 days	700	180
2 weeks	750	150
3 weeks	800	
1 month	1000	
1½ months	1300	
2 months	1400	
2½ months	1600	

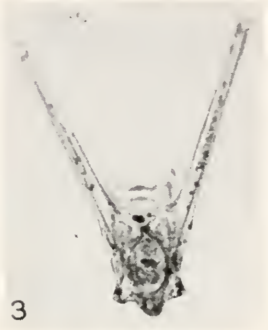
After reaching their maximal size, and often earlier, the arms begin to go to pieces; the flesh peels off, leaving the bare skeleton (Plate IV, Photograph 13). By four months several of the arms have gone to pieces; the body of the adult is conspicuous, but the head of the pluteus remains (Photograph 14). Sometimes the arms are shed as a whole piece like a shell. In Photograph 15 three of the arms shown in the upper Photograph (a) were shed as a unit the following day, as shown in the lower Photograph (b). The animal was left with one arm (Photograph 16). This was thrown off, but the head of the pluteus remained, and the body of the adult with its primitive spines was developing (Photographs 17, 18). The animal at this time was about a half millimeter in diameter. These later stages in metamorphosis took place in my cultures within a few days. The whole process from fertilization to metamorphosis took over four months (July 12 to November 17). I do not know whether this is the normal period under natural conditions as my cultures were subjected to changes in temperature and food. My last four animals all died at this time. They are known to require a different food after metamorphosis, which I did not have at hand. According to Shearer, deMorgan and Fuchs (1914), the best food is the calcareous protozoan Trichosphaerium; and later the red alga Corallina, these furnishing the calcareous matter needed for the development of the test and spines (l.c., p. 276). Miss Gordon (1929) has given a complete account of the further development of the young adult with especial attention to the test.

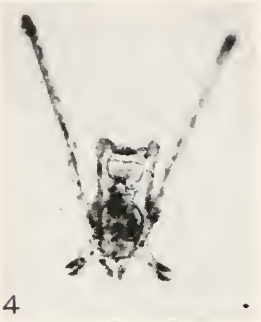
PLATE V


1 Eggs to scale

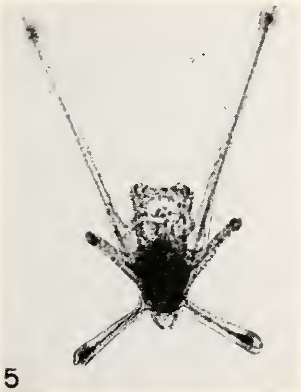

2 3 days

PLUTEI FROM NORMAL EGG

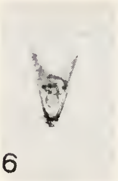

3 10 days

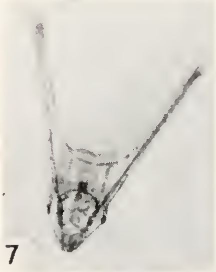

4 11 days

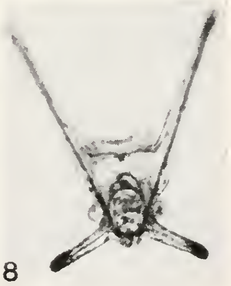
NORMAL


5 3 weeks

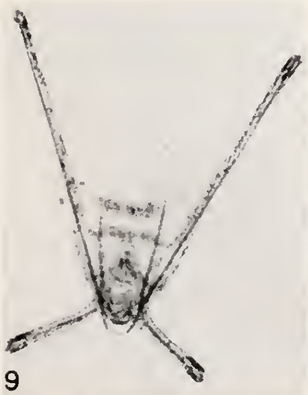
PLUTEI FROM WHITE HALF EGG

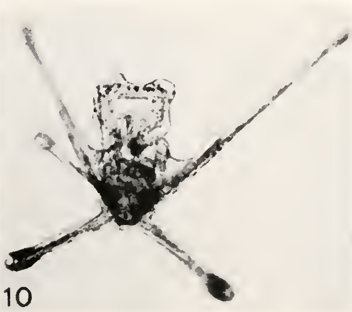

6 3 days


7 10 days


8 10 days

PLUTEI FROM WHITE HALF EGG


9 11 days


10 3 weeks

PLUTEUS FROM CENTRIFUGED EGG

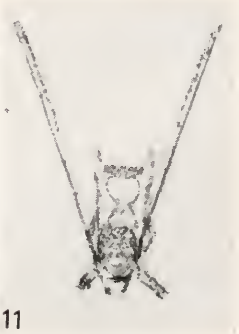
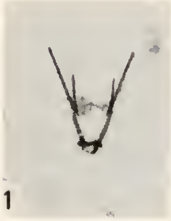
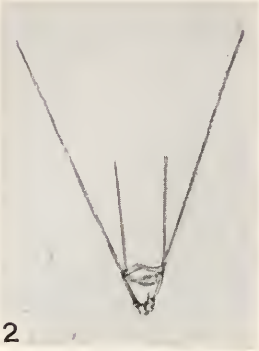

11 2 weeks

PLATE VI

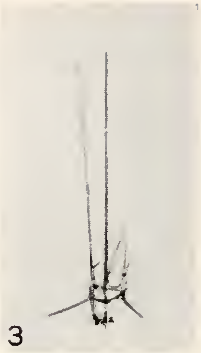
SKELETONS


1

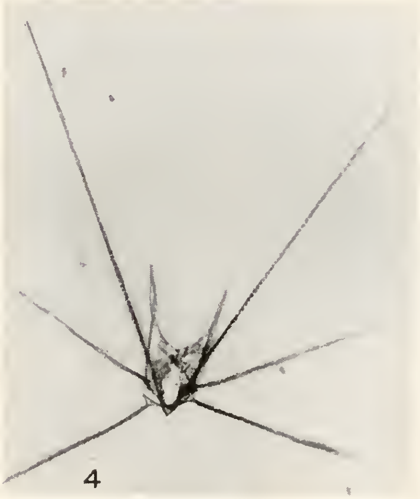
2 days


2

8 days

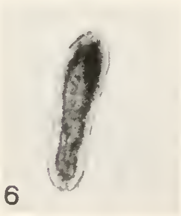

3

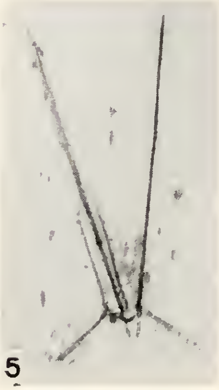
11 days


4

1 month

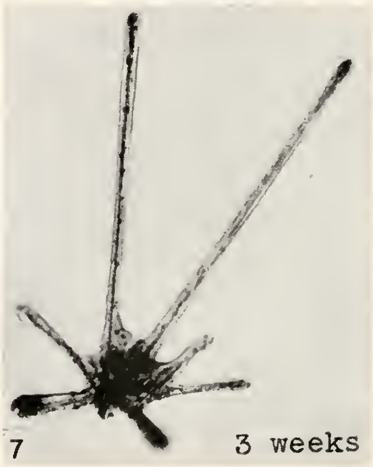
BROKEN OFF ARM


6


5

White half

ABNORMAL


7

3 weeks

The arm skeletons of the pluteus are interesting. The skeleton of the original long pair of (anal) arms is fenestrate, that is, it is a rod with holes in it (Plate IV, Photograph 19). The new pair of red-tipped (ventral lateral) arms have a solid skeletal rod with no holes (Photograph 20). This photograph shows also the concentration of pigment at the tip. The rod in the second new pair of long (dorsal lateral) arms, between the other two long pairs, is again fenestrate (Photograph 21). As is well known, the skeletons of the original long (anal) arms are solid in the plutei of many sea urchins. We have, in general, two types of plutei, those with fenestrate arm skeleton such as Arbacia, Tripneustes, Sphaerechinus and the sand-dollar, Echinarachnius; and those with solid arm skeleton, such as Strongylocentrotus, Lytechinus and Psammechinus. This has been of great value in hybridizing experiments, in determining maternal and paternal inheritance.

Development of the white half egg

The Arbacia egg can be separated into a white and a red half by centrifugal force. The white half when fertilized develops in the same way as the normal whole egg (E. B. Harvey, 1932, 1940). A normal pluteus is formed in two days similar to that from the whole egg except that it is smaller and lacks pigment. The pigment spots, however, begin to come in on the third day after fertilization, and continually increase. The white plutei, like those from the whole egg, do not develop beyond the four armed stage without special feeding. The photographs on Plate V were taken at a greater magnification than the preceding series on Plates III and IV (i.e. approximately 60 times). The eggs to scale are shown in Photograph 1; the normal three day pluteus in Photograph 2. Several later stages of normal plutei are shown in Photographs 3, 4, 5, for comparison with the same stages of the white plutei (Photographs 6–10). The ten day white plutei (Photographs 7, 8) are in all respects like those from whole eggs (Photographs 3, 4). There is the same massing of red pigment in the tips of the first new pair of ventral lateral arms. There is now no difference in size between the plutei from the half egg and those from the whole egg, in fact the former may be larger (Photograph 9). The three weeks pluteus from the half egg (Photograph 10) has the three pairs of long arms like that from the whole egg (Photograph 5). These were not carried any further, but it seems certain that the later development would be like that of the pluteus from the whole egg.

Development of the centrifuged egg

The very young pluteus from the centrifuged egg has the pigment granules concentrated in certain areas, most frequently above the mouth, though they may be in other positions. The pigment spots are also unevenly distributed at first, but after three or four days they are fairly uniformly distributed so that one cannot distinguish between the plutei from centrifuged eggs and those from normal eggs.[2] The later development, when fed, is like that of the normal pluteus. In Photograph 11 is shown a two weeks pluteus from a centrifuged egg, which is similar to the pluteus from a normal egg of about the same age shown in Photograph 4.

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[2] The pigment spots of the plutei are bright red, in contrast to the brownish red color of the unfertilized eggs. However, in centrifuged eggs, the concentrated mass of pigment granules is bright red. This difference in color is well shown in kodochrome slides when the contrasting objects are taken on the same slide. E. G. Ball (*Biol. Bull.*, **97**: 231) has compared the absorption spectra of acid alcohol extracts of plutei and eggs, and has found the two pigments identical.

Skeletons

The skeletons from plutei of various ages are shown in the photographs on Plate VI; the fully formed skeleton is shown in Photograph 4. A skeleton from a white half egg is shown in Photograph 5, quite like that from a whole egg of the same age (Photograph 3).

Abnormalities

The only abnormal later pluteus occurring in my cultures is shown in Photograph 7, on Plate VI. It had one extra arm on one side.

MAGNIFICATION OF PHOTOGRAPHS

The photographs were taken of the living animal with different objectives, as indicated below in brackets, and a 10X ocular.

Plate I. Photographs 1, 2 about 420X (70X). Photographs 3, 4 about 240X (40X). Photographs 5, 6, 7 about 180X (30X).
Plate II. All photographs about 180X (30X).
Plate III. All about 24X (4X).
Plate IV. Photographs 13–18 about 24X (4X). Photographs 19–21 about 240X (40X).
Plate V. All about 60X (10X).
Plate VI. Photographs 1–5 and 7 about 60X (10X). Photograph 6 about 240X (40X).

Explanations of the plates are given on the plates, and in the text.

SUMMARY

1. The growth and metamorphosis of the *Arbacia punctulata* pluteus after the four day stage has been traced by means of photographs. The plutei were raised on the diatom, *Nitzschia closterium*.

2. The pluteus from the white half egg develops pigment spots after the third day, and develops in exactly the same way as that from the whole egg, when fed Nitzschia.

3. The pluteus from the centrifuged, stratified, egg becomes like that from the normal egg after the third day and develops similarly when fed.

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A NOTE ON THE REORIENTATION WITHIN THE SPINDLE OF THE SEX TRIVALENT IN A MANTID

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In most mantids during the formation of the spindle in late meiotic prophase, the kinetochores of each bivalent move suddenly apart towards opposite poles stretching the chromosomes. This is known as the premetaphase stretch stage and is followed first by recontraction of the chromosomes and only then by their gradual congression at the equator to form the metaphase plate. The bivalents move to the equator with no change in their original orientation of one kinetochore to each pole. The behaviour of the sex trivalents, however, both during the stretch stage and the ensuing congression, presents a more complicated situation. As normal behaviour one would expect the X_1X_2 kinetochores to orient towards one pole and the Y kinetochore towards the other, but actually a large number of sex trivalents appear to orient at random. There result, in addition to normal orientation, several types of malorientation. White (1941) first observed such malorientation of sex trivalents; he noted a high frequency of malorientation in 3 species of mantids and concluded that some reorientation must take place before metaphase formation. The conclusive proof for such reorientation was given by Hughes-Schrader (1943), who demonstrated, in *Stagmomantis carolina,* a decrease in the number of maloriented sex trivalents between premetaphase stretch and final metaphase. In the present note are recorded observations on *Hierodula* sp. which show that in this species also reorientation of trivalents takes place.

The material consists of testes fixed in PFA3, from a nymph of *Hierodula* sp., collected near Bombay (India), and placed at my disposal by Professor J. J. Asana to whom I am greatly indebted. Sections ranging from 6 to 10 μ and stained in iron haematoxylin were used for the study.

The presence of a sex trivalent in this species of Hierodula (specific identification is not available) was recorded by Asana (1934) who also established the total number of chromosomes in the male as 27. Later Oguma (1946) recorded the same chromosome complement in the males of four species of Hierodula.

In the present material all sex trivalents in which a lateral view of the spindle is presented during the premetaphase stretch, and again during the metaphase were counted. Those trivalents whose position prevented a positive determination of their orientation were also recorded. During the stretch stage various types of orientation were found. In some cases one of the X's was oriented towards one pole while the other X and the Y were oriented towards the opposite pole; in others the Y was stretched between the two X's while in the rest the orientation was normal. These configurations, assumed by the sex trivalents during the premetaphase stretch, involve a genuine orientation of the kinetochores to the division center comparable to that ordinarily occurring at metaphase. This is shown not only by the position of the chromosomes and their attenuation at the kinetochores but also by the fact that chromosomal fibers are formed between the center and the kinetochore in both maloriented and normally oriented chromosomes.

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* This work was done in the Department of Zoology, Columbia University. I am greatly indebted to Professor Franz Schrader for giving me the facilities to work and to Dr. Sally Hughes-Schrader for suggesting this problem and her helpful criticism.

TABLE I

Orientation of sex trivalent during premetaphase stretch and at metaphase

	Normal	Malorientation			Not analyzable
	X_1X_2 ↕ Y	X_1 ↕ Y ↕ X_2	X_1Y ↕ X_2 X_2Y ↕ X_1	Total	
Premetaphase	42 =65.6 per cent	15	7	22 =34.4 per cent	36
Metaphase	149 =97.4 per cent	3	1	4 =2.6 per cent	0

It will be seen from Table I that during premetaphase stretch as many as 34.4 per cent of all analyzable sex trivalents are maloriented. This number is, however, strikingly reduced to 2.6 per cent in metaphase. Even if all non-analyzable trivalents were assumed to be normally oriented, still the number of maloriented sex trivalents is 22.0 per cent of the total, which is quite significant in relation to the 2.6 per cent of malorientation found at metaphase. This clearly proves the occurrence of reorientation in Hierodula and supports the earlier observations of White and Hughes-Schrader. This conclusion leads us to the basic question of what processes underlie the reorientation. The probable explanation must be sought in the role of the kinetochore. Further investigation of this problem in other species of mantids is planned.

SUMMARY

During the stretch stage in the meiosis of the male Hierodula a high percentage of malorientation of the sex trivalent is found. At metaphase, however, the number of maloriented configurations is so small that a considerable amount of reorientation must occur between these two phases. The forces involved are obviously of some significance in the general problem of the mitotic mechanism.

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MODIFICATION OF THE RESPONSES OF TWO SPECIES OF BUGULA LARVAE FROM WOODS HOLE TO LIGHT AND GRAVITY: ECOLOGICAL ASPECTS OF THE BEHAVIOR OF BUGULA LARVAE

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It is well known that the distribution of sessile organisms is markedly influenced by environmental conditions that affect the setting of their larvae. Thus, oysters are abundant at the mouths of certain rivers where their copper-laden waters are mingled with that of the ocean, and both maxima and minima of settings can be correlated with the amount of copper present during a critical stage of their development. (Cf. Prytherch, 1934.) Furthermore, rather frequent cases of co-occupation of a habitat by communities of sessile organisms of entirely different phyla would suggest the possibility that conditions favorable for the setting of one group are also advantageous to the other. The following observations on the effects of light and temperature on two species of *Bugula, B. flabellata* and *B. turrita,* from the Woods Hole region are presented partly because these factors influence distribution by affecting the attachment, metamorphosis and growth of the larvae and partly for the purpose of comparing and contrasting the behavior of these organisms with that of *B. neritina,* formerly studied at Beaufort, North Carolina (Lynch, 1947). The problem of distribution of two species of the same genus is often a baffling one, as is well illustrated by the fact that *B. flabellata* and *B. turrita* occupy communicating waters not more than 100 yards from each other yet each is found almost exclusively in its own particular habitat. A better understanding of the physiology of the larvae may lead to the beginning of a solution of such problems of distribution.

Contrasting features of the larvae of B. flabellata and B. turrita

Since a description of the larva of *B. flabellata* and its reactions to light and gravity has been given by Grave (1930), only contrasting features of the two larvae or additional details of their behavior will be presented here. *B. flabellata,* the smaller of the two (average, 0.17 by 0.19 mm.), has ten or twelve flagella in its pyriform region and is devoid of light-reactive organs. *B. turrita* is larger (average, 0.19 by 0.20 mm.), has four or five long slender flagella and four brilliant-red, spherical eye-spots, two very close to the pyriform organ and two slightly larger ones located in the opposite hemisphere.[1] The whole body of the larva, except the eye-spots fluoresces faintly in ultra-violet light of 3600 Å. Ejected holdfast material and disintegrated larvae, however, do not fluoresce. This response to ultra-violet light seems to be caused by some substance in the integument of the larva. The cilia at the equator of both species are more active than those in other regions.

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[1] It is difficult to understand why Grave (1930) referred to the light-receptive organs of the larva of *B. turrita* as being "darkly pigmented" and failed to mention their red color. Even with the light cut down to a minimum, their brilliance is an outstanding feature.

The taxonomy of the genus *Bugula* leaves much to be desired at the present time. In the position, color and shape of the eye-spots and in external structure the larva of American *B. turrita* is identical with that of the European *B. plumosa,* but the ground color of the former is yellow or flesh-colored (like that of the European *B. flabellata*), with a faint band of orange pigment at the equator, whereas that of *B. plumosa,* according to Nitsche (1870), is pure white. Furthermore, the larva of European *B. flabellata* differs from the American form, devoid of light receptive organs, since the former has ten symmetrically arranged eye-spots that are figured by Nitsche (1870) as slit-like or elongated and surrounded by fine cilia, but are ovoid according to Barrois' (1877) plates. Grave (1930) noted the difference between American and European species and stated that Calvét (1900) also referred to a confusion of varieties. Is the organism called *B. turrita* in America merely a variety of *B. plumosa?* Or is this a case in which evolutionary changes have affected only the larvae in some instances and only the adults in others? Since two species of *Bugula* sometimes have identical larvae and since the same species apparently may have two different larval forms, it is not unreasonable to suppose that mutations could affect the form of either larva or adult independently of each other.

Materials and Methods

Adult colonies were kept in darkness over night and until the experiments were begun. After exposure of the parental colonies to light, the photopositive larvae released by illumination were easily pipetted to experimental vessels. Generally a single group of adult colonies yielded enough larvae for experiments on several different days. For observations on the geotropism of the larvae and their reactions to light, small vials 1.5 cm. in diameter and 8 cm. high, as well as stender dishes were employed; for microscopic examinations stender dishes and occasionally well slides were used. When slides were used, they were covered to prevent evaporation, which hastens setting by increasing the salinity. A box, 5 by 5 by 15 cm., covered with black paper on all surfaces except the exposed side, was used for experiments on the effects of colored lights.

The Reactions of the Larvae to Light, Heat and Gravity

Diffuse daylight. As described by Grave (1930), the larvae are intensely photopositive during the first three or four hours after their release from the ovicells and then become photonegative. The writer found the photonegative reaction to be somewhat more intense than that described by Grave (1930), who concluded that "it might be overlooked" because of its gradual onset. By 6–8 hours both species of larvae were always definitely photonegative and this reaction was intensified by placing them in sea water diluted by 50 per cent. Furthermore, they changed their reaction from positive to negative immediately after they were placed in sea water buffered to a pH of 9.6 (borate buffer) or diluted by 50 per cent, even when the latter was made hypertonic by the addition of sucrose. In sea water containing 2.5 mg. of copper chloride per liter the majority of the larvae, still photopositive 30 minutes after exposure, did not swim towards the light when the dish was reversed as they normally do. When a concentration of 5 mg. copper per liter was used, they became photonegative within 30 minutes. Even 0.5 mg. per liter reduced the intensity of the photopositive reactions. Since these peculiarities were not observed when the organisms were ejected from a pipette into sea water, mechanical force can hardly be the cause of this reaction. The absence of photic responses in mixtures of sea water and magnesium chloride was mentioned in a previous paper (Lynch, 1949).

Reactions to blue and red light. When a prism spectrum was used to illuminate vials placed horizontally in a black box about 16 feet from a 500 watt bulb, the larvae aggregated densely in the yellow, orange and red regions during their photopositive phase but afterwards congregated in large numbers (80–90 per cent) in the dark region beyond the violet when only the middle portion of the vials was illuminated, or in the violet region when the whole tube was exposed. Apparently this behavior was merely a response to heat, which the larvae tried to avoid, or to light intensity rather than to color.

Since the above method was unsatisfactory, the remaining experiments were carried out with blue and orange-red Eastman Kodak Wratten filters, numbers 76 and 72 respectively. Number 76 transmits a wavelength of approximately 4200–4800 Å and number 72 a wavelength of 5800–6600 Å. These filters have nearly equal relative energy transmission when illuminated with a 400 watt bulb according to Hecht (1921). One end of the vials was illuminated by blue light and the other by red. In the center there were two dark bands caused by the opaque paper of the edges of the two filters where they touched each other. More specimens attached in these dark regions than in either the blue or the red end; these, of course, were not counted in the total number that responded to the colored lights. Of the 292 larvae used in 5 trials 67 per cent attached in the red end. Actually, due to errors in counting large numbers of larvae often attached one on top of the other, the percentage was probably larger than this. In four of the trials there were 1.5 to 2 times as many in the red end. The standard error of the proportion was obtained from the formula, $S.E._p = \sqrt{pq/N}$, for calculating the significance of the results. If it is assumed that the null hypothesis holds in this case, the expected percentages in each end would be 50 per cent (.50), and both p and q would equal .5 each. Thus the $S.E._p = \sqrt{(.5 \times .5)/292} = .0291$. Since an excess of .17 (67 per cent minus 50 per cent) over that postulated by the null hypothesis is at least five times the $S.E._p$, the results would fall easily within the range of "very significant." In these experiments *B. flabellata* showed as much uniformity of response as *B. turrita,* despite the absence of eye-spots on the former. Visscher (1927) obtained somewhat similar results with colored tiles, for more Bryozoa attached to red test panels than to green, black or yellow ones, and no settings occurred on the white ones. (Cf. also Edmondson and Ingram, 1939.)

Darkness. As Grave (1930) had observed, darkness delays fixation and favors attachment to the surface of the water. The writer found the larvae of *B. turrita* to be almost universally active at the end of 24 hours, when kept in complete darkness in a microscope case, and the majority continued to swim for three or four days (the normal duration of the natatory period of most larvae of this species does not exceed 24 hours). Specimens kept in bottles remained active longer than those in uncovered syracuse dishes. Since the sea water in the latter became more concentrated by evaporation, the increased salinity hastened metamorphosis. (Cf. Lynch, 1947). By six days the attached organisms had elongated considerably (maximum length, 1.45 mm.), giving the surface of the water a fuzzy appearance; this was caused by a great abundance of transparent material, much of it in the form of four, symmetrically placed stolons for attachment to the surface film and the remainder organized into a club-shaped structure joined to the stolons and containing in its center an opaque spherical mass that closely resembled the unmetamorphosed larva. The eye-spots were generally visible either in the opaque mass or at a short distance from it in the transparent parts. Apparently development ceased after elongation, for polypides were never observed.

Effects of temperature. Heating the medium to 30° C. accelerated metamorphosis, and raising the temperature to 32–35° C. caused cytolysis; both effects were more pronounced in *B. turrita* than in *B. flabellata*.[2] Many of the former, mere rings of ciliated tissue without material in their centers, were often observed swimming slowly in test tubes exposed to light from a 500 watt incandescent bulb. Enormous amounts of adhesive material always surrounded the larvae after extrusion of the hold fast, but rigid attachment failed to occur. Expansion, especially by elongation along the apico-basal axis, followed exposure to heat. Sausage-shaped streamers of tissue from the pallial furrow, similar to those produced by exposing the larvae to sea water containing an excess of magnesium chloride (Lynch, 1949), were extruded by the larvae of *B. flabellata* from their apical ends, which always looked larger than normal. Development was poor or totally lacking in both species, even when cytolysis did not occur. Marcus (1926) briefly mentioned the accelerating effect of heat on bryozoan larvae.

Geotropism. When diffuse daylight enters a test tube of sea water horizontally, the larvae become fixed at various places along the side farthest from the window, although attachment to the surface is also very common. Under experimenal conditions the larvae generally fell to the bottom just after immersion in a new medium, especially if it contained an excess of various salts. When sea water was mixed with equal parts of normal solutions of sodium, potassium, magnesium or calcium chlorides, the larvae did not swim to the surface again, apparently because ciliary action was too feeble. In mixtures of 80 cc. sea water per 20 cc. of normal calcium chloride, however, vigorous swimming movements were maintained. Heating the medium to 30° C., keeping the larvae in darkness or immersing them in 80 cc. sea water per 20 cc. normal sodium chloride favored attachment to the surface. This concentration of sodium chloride, however, affected the two species somewhat differently. Since *B. flabellata* metamorphosed almost immediately in this mixture most of the larvae did not recover sufficiently from their initial "fright-reaction" to reach the surface, and floating larvae were not often observed; *B. turrita* remained active longer and many were at the surface when fixation took place. · During the photonegative phase the larvae of *B. flabellata* swimming in normal sea water could be made to move downward by placing the light source above them or upward by illuminating them from below. (Cf. also Grave, 1930.) It seems likely, therefore, that in this species the positive geotropism that occurs in nature towards the end of the matatory period is brought about both by light and by a reduction in ciliary movement. In this respect the species at Woods Hole differ considerably from *B. neritina,* for geotropism in the latter is apparently independent of phototropic responses.

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[2] Experiments on *B. neritina* had shown that a reduction of temperature from 23° C. to 7° C. caused all the larvae to become geopositive and lengthened the natatory phase by 2–3 hours (Lynch, 1947, p. 128). Since the writer was not interested in lengthening the larval phase of the Woods Hole species, similar experiments were not repeated. It cannot be assumed, however, that the results would have been similar, since Barrois (1879) reported that a bowl-full of Serialaria (Ctenostomata) that invariably attached at night, was placed in ice and maintained at a temperature near zero all night. Contrary to his expectations, setting took place in a normal manner.

DISCUSSION

The experiments just described and those presented in former papers (Lynch, 1947, 1949) show that heat, light, salinity and the relative proportions of ions in sea water can profoundly affect the natatory period of *Bugula* larvae and the subsequent growth of zooids. From an ecological standpoint, environmental fluctuations that affect the setting of larvae are of paramount importance. Hutchins (1945), having observed that adult species of Bryozoa grew quite well after being transplanted from their natural habitat to one where they were either rare or totally absent, concluded that "in all probability the critical tolerances of environmental variations are those of the larvae, particularly during metamorphosis when they may be supposed to be minimal." An interesting problem is posed by the peculiar distribution at Woods Hole of the two species of Bryozoa under discussion. Grave (1930) stated that *B. turrita* is found in Vineyard Sound, but not in the Eel Pond, whereas *B. flabellata* is abundant in the Eel Pond, but is not ordinarily found outside it, even though the two bodies of water are less than 300 feet apart and communicate freely with one another.[3] Although the adults when transferred from one region to the other on a raft may live for a few months or a year, all efforts to establish new colonies by the transplanted species have so far met with failure (Grave, 1930). A similar peculiar distribution of *Teredo navalis* and of certain hydroids can also be observed. What explanation can be given for these facts? That the larvae are extremely sensitive to the ions present in sea water is evident; whether they can complete metamorphosis and attain normal growth depends upon a very delicate balance of the chemical constituents of their environment. There are, however, so many variables affecting larval behavior that it is extremely difficult to isolate specific ones as causative agents of ecological distribution. Conceivably a particular species thrives best under conditions that enable the organism to terminate larval life after an optimum swimming-time, since larvae that are induced to swim long beyond the normal time of setting rarely develop or form zooids comparable to the controls in size or differentiation. Species such as *B. neritina* with an extremely short natatory period under laboratory conditions seem to be more adversely affected than those whose larval life is of longer duration. It may be that stored nutritive material essential for the formation of zooids is exhausted by prolonged swimming.

The behavior of the two species of *Bugula* from Woods Hole shows certain marked similarities to that of *B. neritina* (from the Beaufort region) and a few striking contrasts. Their reactions to excesses of various metals are nearly identical (Lynch, 1947, 1949). (Copper, however, was not tried on *B. neritina.*) But there are significant differences in their responses to light and gravity. The larvae of *B. neritina* never have a photonegative phase at any time under laboratory conditions, although there is some evidence that they become indifferent to light just before setting. In this respect they resemble the European variety of *B. flabellata* described by Nitsche (1870). Furthermore, they almost universally remain near the surface, and up and down movements in a vial occur but rarely. The larvae of both species from Woods Hole, however, swim vertically along the side of the container during their photonegative phase as readily as they do horizontally during the transitional period when their phototropic responses are beginning to reverse.

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[3] He referred, of course, to the natural habitat of the two species. Actually both species were found by the writer growing on a raft at the entrance to the Eel Pond (August, 1949).

The marked similarity in both phototropic and geotactic behavior of the two species from Woods Hole and the contrast that exists between the behavior of these species and that of *B. neritina* would suggest the possibility that environmental conditions in the two regions might be partially responsible for differences in behavior. Both the extreme brevity of the natatory phase of the Beaufort species and the failure of the larvae to become geopositive at any time under laboratory conditions would seem to be influenced at first sight by two obvious differences in environment, a higher temperature and a brackish condition of the water. This hypothesis is based on the experimental evidence that extreme variability of the duration of the natatory period of a given species can be brought about by altering the ionic balance of the medium, by changing the salinity or by varying the temperature. By altering these factors the geotropic behavior of the larvae can also be changed. Since the abundance of oysters in the brackish waters of Beaufort would suggest the probability that the copper content of this region might be greater than it is at Woods Hole, it would not be unlikely that this ion, capable of hastening the onset of metamorphosis in several sessile organisms, might be largely responsible for the extreme brevity of the larval stage of *B. neritina.*[4] The hypothesis that a greater concentration of copper in the Beaufort sea water and the higher temperature prevailing in that region might play a role in causing contrasts in behavior of the northern and southern species is not illogical, since the natant phase of *B. flabellata* can be shortened appreciably either by adding $CuCl_2$ to sea water or by raising the temperature of the medium. Furthermore, warming the sea water to 30° C. favors surface attachment of *B. flabellata,* whereas cooling the medium causes the larvae of *B. neritina* to become geopositive and prolongs their larval stage; thus, either species may be made to react like the other in this respect. Apparently the pelagic habits of *B.*

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[4] In brackish waters, according to Prytherch (1934), the copper content may reach a concentration of 0.1–0.6 mg. per liter during low tide, whereas it rarely exceeds 0.02 mg./liter in the sea (Galstoff, 1943). Likewise the relative proportions of sodium, magnesium and calcium in brackish waters differ considerably from the distribution of these ions in the ocean, since the order of concentration of these ions approaches that of fresh water (calcium, magnesium and sodium). (Cf. Clarke, 1924.) Recently (after this paper had been prepared in its present form) Glaser and Anslow (1949) gave the copper content of Woods Hole sea water (spectroscopically determined) as $2.50 \pm \times 10^{-7}$ M Cu. They found that a sample of Beaufort sea water had a value as high as 1×10^{-6} M Cu; they noted, however, that the latter may have been contaminated (p. 127 and 128).

neritina are correlated with their brief natatory period (Lynch, 1947);[5] likewise the much more frequent occurrence of geopositive settings of *B. flabellata* under normal conditions seems to be related to their naturally longer larval phase, for the number on the bottom of a vessel begins to increase after the larvae have been active for several hours.

Nevertheless, even though the behavior (except phototropism) of one species can be duplicated almost exactly in the other by altering the environment, neither differences in temperature *alone* nor in the content of the sea water can account for contrasts in the behavior of the northern and southern species. The monthly mean temperatures during July and August at Woods Hole and at Beaufort differ by only six or seven degrees, according to McDougall (1943), and experiments performed at Beaufort by the writer showed no marked change in the behavior of *B. neritina* when the temperature was reduced to 21° C. (the monthly mean for Woods Hole), although a more drastic reduction of temperature did reverse the geotropism of the larvae and prolong their free-swimming phase. Likewise, when larvae of *B. flabellata* were immersed in sea water taken at low tide from the Beaufort region and shipped to Woods Hole, their behavior was like that of the controls. No shortening of the larval phase was observed. (The experimental sea water had a pH of 7.5 when used.) Logically, negative results were partially predictable, since previous experiments had shown that a concentration of copper chloride as low as 0.5 mg per liter of sea water (about maximum for brackish waters) had no appreciable effect in shortening the natatory period, although higher concentrations were effective.[6] Is it then a mere coincidence that the southern species with its short natant phase lives in an environment where two factors, a higher temperature and a presumably (?) greater concentration of copper, are both present and either of these can accelerate the onset of metamorphosis? There are two possibilities. On the one hand, since experimental modifications of temperature that proved to be effective in lengthening or shortening the larval phase were more drastic than those actually prevailing in nature and since the same was true of the copper content, conceivably slighter changes of both factors combined might be as effective as more extreme alterations of each one separately. It should be noted that, since the temperature of the Beaufort sea water was not raised to the degree ordinarily prevailing in that region, the environment of the southern species was only partially duplicated on the northern one. On the other hand, since there are generic and specific as well as individual differences in the natatory phase of bryozoan larvae, it seems more tenable to assume that species genetically determined to have a short larval period can thrive only in an environment where ions, presumably requisite for setting, can be rapidly absorbed. Conceivably, there may be specific differences in the ion-absorbing ability of larvae. This assumption, however, offers no explanation for the fact that the larval phase of the majority of a given species may end at two hours on one day and at ten hours on another.

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[5] By using two sets of data that appeared to have the least positive correlation, the coefficient of correlation between the number of larvae of *B. neritina* that were kept active for four hours by a reduction of salinity and the number that became geopositive at the time of setting was found to be + .53 and + .57.

[6] It should be noted that concentrations of $CuCl_2$ as high as 1.25 mg. per liter had no effect; Prytherch (1934) found that in his experiments virtually all the copper was precipitated when less than .5 mg./liter was used.

(See Table I, p. 30, Lynch, 1949).[7] Before the relative effects of environment can be evaluated it is necessary to know whether there are significant differences in the length of the natatory period of the same species in different localities. Since Edmondson and Ingram (1939) reported that larvae of the Hawaiian *B. neritina* attach at night as readily as during the day, it may be that these organisms have a much longer natant phase than the ones studied at Beaufort. The latter (laboratory conditions) were always released a short time after exposure of the parental colonies to light, and active unattached ones could rarely, if ever, be found after noon. Caution must be observed, however, in making such an assumption, for it is unwise to conclude that the natatory period of larvae under natural conditions is as brief as it is in the laboratory. Indeed, the vertical distribution of adults indicates that in nature these larvae are probably active longer than an hour or two (maximum time in the laboratory) and undergo sufficient activity to make most of them geopositive. It would be extremely valuable to have definite information regarding the behavior of *B. neritina* on the California coast. There is also need for further research on the interaction of factors capable of accelerating or retarding metamorphosis. Some may have antagonistic effects; others may act synergistically.

The writer is indebted to Professors J. H. Bodine and H. W. Stunkard for reading the manuscript and to Dr. M. D. Rogick for information regarding the taxonomy of the genus *Bugula*.

SUMMARY

1. The larvae of both *B. flabellata* and *B. turrita* are photopositive in diffuse light during the first 3–4 hours after release from the ovicells and then become photonegative. They became photonegative immediately, however, when they were placed in sea water buffered to a pH of 9.6 or diluted by 50 per cent; sea water containing copper chloride either reduced the intensity of the photopositive phase or caused a reversal of phototropism depending on the concentrations that were used. These organisms became indifferent to light in mixtures of 80 cc. sea water per 20 cc. of either normal calcium chloride or magnesium chloride.

2. Larger numbers of larvae attached in the red end of a test tube illuminated by red and blue light passing through Wratten filters than in the opposite end.

3. Heating sea water to 30° C. hastened metamorphosis and favored surface attachment, but development was poor or entirely lacking at this temperature. Darkness delayed metamorphosis and also caused attachment to the surface film;

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[7] Barrois (1879) stated that in the laboratories at Roscoff the same species of Bryozoa generally showed extreme variations of the natatory period on different days and that cases of this strange phenomenon could be found in all groups but was especially striking in *Pedicellina* and *Cyphonautes*. In some cases (*Flustrella hispida*, especially) he found it impossible to obtain a single fixation during a period of many weeks, even though the larvae were very abundant. At other times, under apparently identical conditions, fixations took place in large numbers. He noted that the incapacity of larvae to fix themselves might persist for a long time or cease suddenly. At times settings occurred in various parts of the bowls; at other times they took place *en masse* at certain points. But these anomalies were entirely absent in bowls prepared at the same time and place and under identical conditions. Harmer (1922, p. 513), likewise, noted the difficulty of persuading larvae to attach under laboratory conditions and stated that it could be surmounted by placing adult colonies in a vessel closed with fine muslin and left attached to a buoy or placed in a deep tide-pool. Evidently environment is extremely important.

development ceased after a fair amount of growth and a slight degree of differentiation.

4. In mixtures of equal amounts of sea water and normal solutions of sodium, potassium, magnesium and calcium chlorides the larvae became geopositive on entering the medium and remained so during the experiments.

5. Some ecological problems of the distribution of three species of *Bugula* are discussed and tentative suggestions for their solution are offered.

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THE RESISTANCE OF SCIARA (DIPTERA) TO THE MUTAGENIC EFFECTS OF IRRADIATION

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On the basis of the irradiation studies made on Sciara to date, a condition has been noted which is of general interest with reference to the mode of action of x-rays on the hereditary material, namely, an apparent resistance to the mutagenic effects of irradiation. Whereas gross and minute chromosome rearrangements are induced in treated germ cells, visible mutations appear at a negligible frequency.

Sciara is very unusual in this respect. In other organisms, including Drosophila, maize, and Neurospora, x-rays are found to induce both mutations and chromosome breaks at frequencies proportional to the dosage. During the past twenty years at least eight investigators have independently looked for visible mutations among the progeny of irradiated Sciara; altogether only twenty-four or twenty-six mutant characters have been obtained in the several species treated. Unfortunately, the irradiation data are not tabulated in such a way that an estimate can be made of the number of germ cells exposed or the total progeny examined. But certain of the studies were extensive. Both male and female germ cells were treated at various times in the developmental cycle and dosages from 3000 to 30,000 r. applied.

In view of the low mutation rate obtained repeatedly (see Metz, 1938), the chromosomes of Sciara were believed to be resistant to irradiation. The first clue to the contrary was the discovery of a reciprocal translocation in the salivary gland nuclei of larvae taken from cultures of the "Stop" mutant (Crouse and Smith-Stocking, 1938). The salivary gland chromosomes were then utilized in a cytological analysis of F_1 larvae derived from irradiated sperm or oocytes (Metz and Boche, 1939); following exposure of sperm to 5000 r., approximately 25 per cent of the F_1 showed gross chromosomal rearrangements. This unexpected induction of chromosome aberrations in Sciara was confirmed in subsequent experiments.

The experiments reported in this paper were performed in connection with cyto-genetic studies on the unusual behavior of the sex chromosome of Sciara. The data bear on the problem at hand, however, and will therefore be discussed in this relation.

Preliminary data on dominant lethal induction are in line with the rearrangement studies and provide further evidence that the chromosomes of Sciara are sensitive to irradiation. In one experiment designed to pick up X-translocations in *S. coprophila*, nine females were bred singly to adult males which had been x-rayed at 4000 r. The nine females yielded 108 total offspring, while nine control females from the same stock (isogenic) produced 723 total offspring. The exact probability (as measured by chi-square) of this difference is 0.0004. On the basis of this small but very carefully conducted experiment, only 15 per cent emergence was obtained, a dominant lethal value which is practically identical to that measured by Demerec and Fano (1944) in Drosophila sperm x-rayed at 4000 r. It is of interest to note that in the Sciara experiment cited, nine of the 108 survivors (8 per cent) were heterozygous for X-translocations but none transmitted sex-linked visible mutations.

If dominant lethals are regarded as the result of certain types of chromosomal aberrations (see Pontecorvo, 1942), Sciara and Drosophila chromosomes respond to irradiation in a similar manner, and the physiological result (i.e., the lethal phenotype) is the same in both genera. It is with respect to less drastic physiological changes—namely, hereditary alterations classified as "visibles"—that the two genera differ.

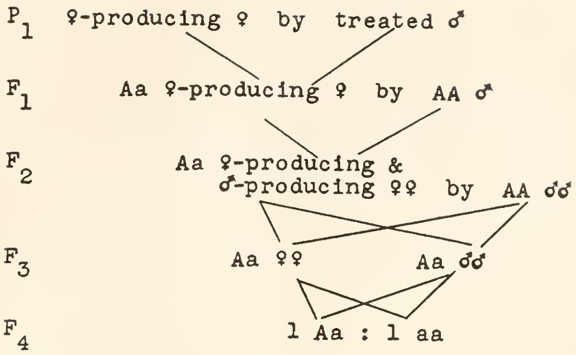
Several factors may account for the low visible mutation rate in Sciara.

(1) The external appearance of this fly (bristle pattern, pigmentation, etc.) is such that only the most conspicuous visible changes are likely to be detected.

(2) There is a distinct difference between the autosomal and the sex-linked mutations, which suggests that the induced mutation rate in this genus is considerably greater than the detected mutation rate. In *S. coprophila,* the species which has been most thoroughly worked, nine autosomal and five sex-linked mutations have been recovered. Of the autosomal group, seven are dominant and two are recessive; among the sex-linked factors, on the other hand, four are recessive and only one is dominant. The exact probability (as measured by chi-square) of this difference is 0.126. In material such as Drosophila, Habrobracon and maize, the dominant mutations constitute a very small percentage of the total number of visible mutations. The relatively high proportion of dominants in Sciara has been interpreted as evidence that this genus is unique in its response to irradiation (Metz, 1938). Such an interpretation is probably not valid, since, as noted above, the dominants constitute a majority only in the case of the autosomal factors. Most likely the discrepancy in the autosomal mutations has a relatively simple explanation, namely the extraordinarily tedious and inefficient technique available for the detection of autosomal recessives in this genus.

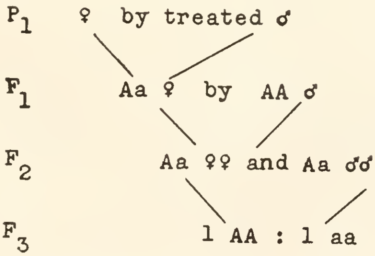
The mode of inheritance and the mechanism of sex determination in Sciara make it difficult to pick up autosomal recessive mutations. *S. coprophila* is monogenic. This means that the females produce either sons or daughters but not both; consequently, an F_1 female derived from an irradiated sperm will yield a family of sons or a family of daughters. If she is a male-producer and heterozygous for an induced sex-linked recessive, half of her sons should show the mutant character. Sex-linked recessives, therefore, can be fairly readily detected in Sciara in the F_2 generation. Autosomal recessives, on the other hand, are practically impossible to pick up because of the monogenic condition described above and because Sciara males transmit only the genes they inherit from their mother; the paternally derived chromosomes are eliminated at the first spermatocyte division. In searching for autosomal recessives, the best procedure is to take the mutated gene (a mutation induced in treated sperm), through the female germ line according to the scheme outlined below.

In order to detect the recessive mutation, a, four successive generations of flies (approximately four months at 70° F.) have to be produced as outlined; and then


P_1 ♀-producing ♀ by treated ♂
F_1 Aa ♀-producing ♀ by AA ♂
F_2 Aa ♀-producing & ♂-producing ♀♀ by AA ♂♂
F_3 Aa ♀♀ Aa ♂♂
F_4 1 Aa : 1 aa

the probability that the mutation will be detected in the F_4 is less than $\frac{1}{16}$. The calculation, of course, does not include I, the probability of induction of the mutation. In the sex-linked mutant strains of *S. coprophila,* male- and female-producers are phenotypically distinguishable. If one of these mutant strains is used in the experiment diagrammed above, the chance of selecting a ♀-producing female and a ♂-producing male of genotype Aa in the F_2 is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ (F_2 females are AA or Aa). Then the chance of selecting a female and a male of genotype Aa in the F_3 is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$, making the final probability $I \times \frac{1}{4} \times \frac{1}{4}$. Thus, the odds against detection of an autosomal recessive in *S. coprophila* are at least 15:1.

In digenic species like *S. reynoldsi* (the females produce both sons and daughters), autosomal recessives can be detected in the F_3 generation according to the following scheme:



In this case the chance of selecting an F_2 male and female of genotype Aa is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$, making the odds against detection of the mutation only 3:1 instead of 15:1.

The evidence presented so far is consistent with the view that the detected mutation rate in Sciara is considerably less than the induced rate. Even so, the induced rate appears to be less than that observed in Drosophila or Habrobracon; for, in the case of the sex chromosome of *S. coprophila,* where recessives can be picked up in the F_2, only five factors (four recessive and one dominant) have been found.

This dearth of mutants is particularly significant in view of the fact that over a period of several years the author has tested a large number of irradiated sperm (approximately 500) and oocytes (approximately 100) of *S. coprophila* in a search for reciprocal translocations which involve the X chromosome (Crouse, 1943 and unpublished). In these experiments, breeding tests were carried to the F_2 generation or beyond; fourteen analyzable (genetic and cytological) X-translocations have been recovered but not a single visible sex-linked or autosomal mutation. Similar experiments conducted on *S. reynoldsi* yielded four X-translocations and no mutations among irradiated sperm (approximately 200 sperm tested).

(3) The presence in the germ cells of *S. coprophila* of large amounts of heterochromatin, in the form of the limited chromosomes, may actually retard the mutation rate. There are no published data on the effects of heterochromatin on general mutability either in corn or Drosophila—two forms in which the B-type chromosomes and the Y, respectively, might well be exploited. Unpublished studies of Miss Jean Kerschner, on the effects of an extra Y chromosome on the x-ray induced visible and sex-linked recessive lethal rates in *D. melanogaster* males, indicate that the extra Y brings about a significant decrease in these mutation rates.

(4) Finally, it is conceivable that, as compared to Drosophila or Habrobracon, the biochemical pathways which are available in Sciara result in a more restricted range of phenotypic variability.

Summary

Several facts have been discussed which may contribute to the apparent resistance of Sciara to the mutagenic effects of irradiation. New data are presented which (1) support the view that the visible mutation rate induced in this genus is lower than that found in Drosophila or Habrobracon; and (2) reveal a high dominant lethal mutation rate in this genus.

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RECOVERY FROM ULTRA-VIOLET LIGHT-INDUCED DELAY IN CLEAVAGE OF ARBACIA EGGS BY IRRADIATION WITH VISIBLE LIGHT[1]

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Kelner has reported that exposure of *Streptomyces griseus* conidia to intense visible light (V.) after inactivation with ultra-violet light (U.V.) enabled these conidia to grow (Kelner, 1949). Dulbecco obtained similar results when bacteria containing U.V.-inactivated bacteriophage were given visible light (Dulbecco, 1949). The experiments to be described show that a similar phenomenon occurs when *Arbacia punctulata* eggs fertilized with U.V.-treated sperm are exposed to intense visible light.

METHODS

The U.V. source was a Westinghouse Sterilamp W.L. 782, 80 per cent of whose radiation was at $\lambda = 253.7$ mμ. The visible light was produced by two 1000-watt tungsten filament lamps, the light from each being focused through a round, water-filled, one-liter pyrex flask onto a white tile. An infra-red filter was placed 5 inches from the illuminated spot which was 2 inches in diameter. A fine-grained ground-glass screen was placed 2 inches above the tile in order to diffuse the filament images. The U.V. intensity, measured with a Hanovia Ultraviolet meter, was 52 micro-watts per cm^2 at the position where the biological material was irradiated. The visible light intensity on the white tile, as measured with a Leeds-Northrop Macbeth Illuminometer, was approximately 28,000 foot candles. The rise in temperature of the irradiated suspension, as measured with a thermometer immediately after irradiation, was less than 0.2° C. The heat generated by the lamps was dissipated by circulation of air with an electric fan. With windows and doors of the laboratory kept open, there was no appreciable rise in the temperature of the room.

Sperm suspensions were prepared by dispersing 2 drops of "dry sperm" in 250 ml of sea water ("0.4 per cent sperm"). Egg suspensions contained 5–10,000 eggs/ml. For irradiation with either V. or U.V., 2 ml of either suspension was pipetted into a pyrex petri dish (without cover) 2 inches in diameter, which formed a layer about 1 mm high. To fertilize eggs, 0.2 ml of the sperm suspension was added to the 2 ml egg suspension with agitation.

The time for cleavage was obtained by counting the number of cleaved and uncleaved eggs, the total being 50 to 100 eggs each time. After determining the point at which 50 per cent of the eggs cleaved, they were again counted to determine total cleavage. Data were discarded unless the controls showed a final cleavage of 95 to 100 per cent.

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[1] Supported by a grant from the U. S. Public Health Service.

Temperatures were recorded but not controlled. In all experiments, temperature fluctuations were less than 1° C. in the course of the experiment.

RESULTS

1. *Time for cleavage as a function of U.V. dose*

Sperm were irradiated for periods from 0.5 to 4.0 minutes and unirradiated eggs fertilized 8 to 10 minutes later. Since at the higher doses cleavage did not reach 50 per cent, comparison was made at the 10 per cent cleavage point. The following table gives the results obtained:

(24° C.)

Exposure time (min.)	0	0.5	1.0	2.0	3.0	4.0
Time to 10 per cent cleavage (min.)	47	88	113	157	214	235

Eggs were treated with U.V. and then fertilized by normal sperm with results as shown below:

(24° C.)

Exposure time (min.)	0	0.5	1.0	2.0	3.0	4.0
Time to 50 per cent cleavage (min.)	47	49	58	62	68	72

2. *Growth of the U.V. effect*

Sperm and egg suspensions were irradiated with U.V. and allowed to stand for varying lengths of time before fertilization. The sperm was irradiated for 0.5 minutes, the eggs for 5.0 minutes. The following results were obtained when irradiated sperm was used to fertilize normal eggs and irradiated eggs were fertilized with normal sperm:

Time for 50 per cent cleavage (25° C.)

T	2	10	20	30	40	50	60	80	c	c′
Irradiated sperm	98	100	98	104	107	110	120	124	44	47
Irradiated eggs	69	71	69	68	68	68	73	70	47	47

T = time in minutes from irradiation to fertilization
c = non-irradiated eggs and sperm. Fertilization immediately after preparation of suspensions.
c′ = non-irradiated eggs and sperm. Fertilization 75 minutes after preparation of suspensions.

There was an appreciable growth of the cleavage delay factors in the irradiated sperm, but there was neither growth nor decay of these factors in the egg.

3. *Irradiation with ultraviolet and visible light before fertilization*

To determine whether V. given to either gamete before or after U.V. irradiation altered the U.V.-induced cleavage delay, eggs and sperm were separately irradiated. The irradiated sperm were used to fertilize normal eggs and irradiated eggs fertilized with normal sperm. The results are given below:

Time for 50 per cent cleavage (25° C.)

	Exposure time						
	V.	U.V.	Control	U.V.	V.	V. then U.V.	U.V. then V.
Sperm irradiated	3′	10″	46	70	46	69	68
	3′	30″	46	117	—	114	109
	3′	2′[2]	41	139	—	135	133
Eggs irradiated	3′	2′	48	72	47	72	74

Visible light given to either gamete did not change the cleavage time. Visible light given before or after U.V. did not significantly shorten or prolong the U.V.-induced delay in cleavage.

4. *Visible light given to the zygote*

When visible light (3 minute exposure) was given to eggs fertilized with untreated sperm at various times after fertilization, the time for cleavage was not significantly affected. The following shows the results obtained:

V. – 3′ *t* – 27° C.

I	2	5	10	15	20	30	c
T	41	41	40	43	42	44	44

I = Time of irradiation with visible light in minutes after fertilization. I is measured to the time of beginning of irradiation.
T = Time in minutes for 50 per cent cleavage.
c = Control not exposed to light.

However, when the zygote was exposed to visible light after fertilization with U.V.-treated sperm, there was marked reduction in the time for cleavage. The following are results from three runs at different times to indicate the degree of reproducibility. In all cases the eggs were fertilized within 10 minutes after U.V. irradiation of the sperm. Repetition of these experiments several times gave essentially the same results:

U.V. – 0.5′, V. – 3′

I (26° C.)	1.5	5	9	11	15	20	30	40	60	c′	c
T	60	58	56	54	69	81	91	94	98	97	44
(27° C.)	2	6	10	35	50					c′	c
T	65	60	56	85	90					97	43
I (25° C.)			10	50	60	70	80			c′	c
T			59	87	90	93	95			96	44

I, T, and c as above.
c′ = eggs fertilized with U.V. treated sperm but not exposed to visible light.

The delay in cleavage (inactivation) is considerably shortened when exposure to V. begins as early as 1.5 minutes after fertilization. This shortening of the cleavage-delay time will be referred to as photoreactivation. Maximum efficiency in photoreactivation was observed when exposure to visible light began at 9 to 11 minutes after fertilization. Detectible photoreactivation was observed as late as 40 to 50 minutes after fertilization.

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[2] In this series, values are for time to 5 per cent cleavage.

Neither U.V. inactivation nor photoreactivation had an appreciable effect on the time for the second cleavage. Below are given the observed times between first and second cleavages after U.V.–0.5′ and V.–3′ at the times indicated:

I	10	50	60	70	80	c′	c
T[3]	26	30	32	32	30	32	26

I, T, c′, c as above.

It was noted in some experiments that c′, and the samples receiving V. at times later than 20 minutes after fertilization, had a considerable percentage of the eggs with a 3 to 4-cell first cleavage. This occurred at about the same time that the remaining cells showed the usual 2-cell cleavage. These 3 to 4-cell first cleavages were absent or rare in c and in eggs receiving V. at 10 to 20 minutes after fertilization. In these cases when allowance was made for the percentage of 3 to 4-cell zygotes by first cleavage, and the time determined for 2-cell zygotes to reach the 4-cell stage, the results were essentially the same as those given above. The factors responsible for the production of 3 to 4-cell first cleavages were not investigated.

5. *Quantity of light for maximum photoreactivation*

Following irradiation of sperm with U.V. for 0.5′, eggs were fertilized and the fertilized eggs exposed to the visible light, exposure beginning at 10′ after fertilization in all cases. The exposure time was varied with the results shown below:

t – 24° C.

E	10	30	60	120	180	300	c′	c
T	87	77	75	78	63	66	99	58

E = exposure time in seconds.
T, c′, c as above.

The data show that exposure for periods longer than 3 minutes did not produce greater photoreactivation.

6. *Photoreactivation in nucleated white halves of Arbacia eggs*

Eggs were centrifuged at approximately 10,000g for 10 minutes and the nucleated white halves and non-nucleated red halves collected separately. These were then fertilized with U.V. treated sperm and the zygotes exposed to visible radiation with the following results for the white halves:

U.V. – 0.5′, V. – 3′ *t* – 26° C.

I	10	20	30	50	65	c′	c
T	72	83	95	100	101	106	46

I, T, c′, c as above.

Photoreactivation was, therefore, possible in the non-pigmented half-eggs although the capacity for photoreactivation appears to be lower than in the whole egg. Since cleavage in the red halves was rare, the presence or absence of the capacity of these pigmented half-eggs for photoreactivation was not investigated.

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[3] Time measured from 50 per cent first cleavage to 50 per cent second cleavage.

7. *Photoreactivation efficiency of light of different wave-lengths*

Wratten filters, 3″ × 3″, were mounted on the ground glass screen with the edges held by scotch tape, and oriented so that all of the beam passed through the filters. With filter No. 73, which transmitted at 560 to 620 mμ (maximum 570 mμ) and to some extent at 680 to 700 mμ and had a total transmission of 1.6 per cent, the following results were obtained:

U.V. – 0,5′, V. – 8.0′ t – 24° C.

I	10	20	60	c′	c	c″
T	80	94	107	106	50	57

I, T, c′, c as above.
c″ = full visible spectrum for 3′ at 10′ after fertilization.

Filter No. 76, which transmitted at 330 to 470 mμ (maximum at 440 mμ) and very little at 690 to 700 mμ with total transmission at all wavelengths of 0.1 per cent, gave the following results:

U.V. – 0.5′, V. – 8.0′ t – 24° C.

I	10	20	60	c′	c	c″
T	80	94	126	120	51	57

I, T, c′, c, c″ as above.

The same amount of photoreactivation was obtained with either filter. Since the total transmission of No. 76 was less than that of No. 73 by a factor of 16, the photoreactivation efficency of the band 330 to 470 mμ was much greater than light of wavelengths 560 to 620 mμ. The intensity of the filtered light was too low to obtain maximum photoreactivation in both cases, but this does not affect the validity of the above deduction.

8. *Time for the "streak" stage*

In addition to the time for 50 per cent cleavage, the time when the "streak" stage first appeared was also noted. In 6 experiments, this time varied from 13 to 18′, but in each case the time was the same, within one minute, for the non-irradiated controls and those which had received U.V.-treated sperm. Since the streak represents the development of the sperm aster, U.V.-irradiation apparently affects a later stage.

9. *Treatment with chemical agents* [4]

Streptomycin, in concentrations of 1 μg/ml to 10 μg/ml, had no effect on cleavage time of eggs fertilized with normal sperm. When sperm were given U.V. in the presence of streptomycin (1 mg/ml), there was no significant change in either U.V.-inactivation or photoreactivation.

Treatment of eggs with adenosine (20 μg/ml), prior to fertilization with U.V.-treated sperm, did not alter cleavage time, nor did it affect photoreactivation. Addition of adenosine to sperm immediately after U.V.-irradiation and fertilization 20′ later, also had no effect.

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[4] We are indebted to the Lederle Laboratories for the folic acid and 4-amino-n-methyl folic acid, the Merck Laboratories for the streptomycin, and the Wellcome Research Laboratory for the 2,4-diamino-5-p chlorophenoxypyrimidine.

Eggs treated with folic acid at concentrations up to 10 μg/ml for 20′ before fertilization, or at 7′ after fertilization, with U.V.-irradiated sperm, showed no change in cleavage-delay time.

Eggs treated with 4-amino-n-methyl folic acid (10 μg/ml), 5′ after fertilization and for 20′ before fertilization, with U.V.-treated sperm, showed no effect on cleavage delay or photoreactivation.

Eggs were also treated with the purine antagonist 2-4-diamino-5-p-chlorophenoxypyrimidine (Falco and Hitchings, '49) for 30′ before fertilization with normal and with U.V. treated sperm. No effect was observed on the normal cleavage time, the U.V. inactivation or photoreactivation.

When U.V.-irradiated sperm were treated with riboflavin (20 μg/ml) or with riboflavin (20 μg/ml) plus adenosine (20 μg/ml) for 15′ before their use in fertilization, there was no effect on cleavage delay. Neither of these substances nor both given together affected normal cleavage time. When sperm treated with riboflavin were exposed to the visible light, they showed first a peculiar rapid vibratory motion and then complete cessation of motion within 10 minutes. Eggs could be fertilized with such sperm immediately after exposure to light. When eggs were thus fertilized, the time for cleavage was prolonged much beyond the usual U.V.-delay time. The cells attempting to cleave became very much elongated and twisted, but were only partially constricted. However, eggs treated with riboflavin for 20′ before fertilization with U.V.-irradiated sperm and not exposed to visible light, cleaved normally and showed no change in the cleavage delay time.

Discussion

Kelner (1949) and Dulbecco (1949) have both suggested that photoreactivation may be a general biological phenomenon. The experiments described here indicate its presence in an organism very different from either of those previously studied. It is interesting that in this respect the combination bacterium-phage is analogous to the combination egg-sperm. This analogy suggests that it would be desirable to determine whether inactivation of the phage could be obtained by U.V. irradiation of the bacterium prior to infection, and whether photoreactivation then follows the same course as in the case of U.V.-irradiated phage.

The present experiments show that there is a critical stage in the interaction of the sperm and egg when susceptibility to photoreactivation is at a maximum. The experiments on the time for the second cleavage also show that the cleavage-delaying conditions or agents are removed by the end of the first cleavage. The delay in cleavage is not due to inhibition or delay in the formation of the sperm aster. The effect must therefore be on some later stage. Once the cleavage constriction begins, the time for completion of cleavage is not significantly different in eggs receiving normal or U.V.-treated sperm within the dosage limits used in these experiments. The overall delay may therefore be attributed to a delay in the onset of prophase or to a prolongation of the prophase-metaphase time, or both. Since maximum sensitivity is at the time of onset of prophase, this appears to be the stage at which the U.V. inhibition becomes effective.

The agent or condition produced by U.V. remains unaltered in the unfertilized egg but increases with time in the sperm, suggesting that stabilizing factors present in the egg are absent in the sperm, so that reactions initiated by U.V. absorption are much retarded or stopped in the egg but not in the sperm. The Arbacia egg contains a very considerable amount of ribonucleic acid in its cytoplasm (Schmidt, Hecht and Thannhauser, 1948 and Brachet, 1933). Consequently the relative inefficiency of U.V. irradiation of the egg may be taken as an indication that U.V.-alteration of ribonucleic acid of the cytoplasm is not involved and that the system affected is in the nucleus. The occurrence of photoreactivation in the non-pigmented half-egg indicates that the pigment is not involved in this reaction; and since most of the granules are removed, the latter also are probably not involved.

The relatively high efficiency of light of wavelengths in the region 360 to 460 mμ, suggests the possibility that intracellular substances with absorption bands in this region may somehow be related to the photoreactivation phenomenon. Riboflavin and folic acid both have absorption bands in this region (Warburg and Christian, 1938 and Stokstad, Hutchings and Subbarow, 1946). However, folic acid given to eggs or sperm does not alter the U.V.-inactivation. Also the folic acid inhibitor, 4-amino-n-methyl folic acid, does not prolong cleavage of the normally fertilized egg nor alter the U.V.-induced cleavage delay. The purine antagonist, 2-4-diamino-5-p chlorophenoxypyrimidine, also has no effect. No definite conclusion can be drawn from the failure to observe effects with any of these substances, since there is no proof that they penetrated the eggs or sperm. An exception may be made in the case of riboflavin since a marked effect was observed when the eggs or sperm were exposed to light. However, the effects observed with riboflavin-treated irradiated sperm seem to be unrelated to photoreactivation. Eggs treated with riboflavin, and U.V.-irradiated sperm similarly treated, show the usual cleavage delay time. The prolongation of cleavage, and abnormalities thereof produced by sperm irradiated with visible light in the presence of riboflavin, may be attributed to toxic photodecomposition products of riboflavin.

CONCLUSIONS

1. Irradiation of either sperm or egg with ultraviolet light will delay the time for the first cleavage division of the zygote formed by such gametes. Since irradiation of the egg is much less effective, the U.V.-inactivation is probably produced in the nucleus. The time of the formation of the sperm aster is not affected.

2. There is no decay or growth of the U.V. effect with time, after irradiation of the egg; but in the sperm there is definite growth.

3. Irradiation of either gamete with intense visible light, after U.V. irradiation, does not alter the cleavage delay.

4. Irradiation of the zygote with intense visible light reduces the cleavage-delay time. The zygote is most reactive at 10 minutes after fertilization, which is approximately the time of onset of prophase, although detectible response may be observed as late as 50 minutes after irradiation. The mitotic stage which is delayed and which responds most to the visible light is the onset of prophase.

5. Treatment with adenosine, streptomycin, folic acid, and 2-4-diamino-5-p chlorphenoxypyrimidine had no effect on either U.V.-inactivation or photoreactivation.

6. Treatment of the egg or zygote receiving U.V.-inactivated sperm with riboflavin, had no effect on the cleavage time. Irradiation of sperm in the presence of riboflavin with visible light, had a toxic effect on the sperm and on the zygote formed from such irradiated sperm.

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TRACHEAL FILLING IN SCIARA LARVAE

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INTRODUCTION

During insect development the gas-filled tracheae of a given instar become enclosed in larger, liquid-filled, coaxial tubes which are to form the tracheal system of the next instar. At molting the old tracheae are withdrawn and shed with the body cuticle. The new system then fills with gas, either immediately or within a short time, the liquid probably passing through the tracheal wall into the blood or tissues (Weismann, 1863; Palmén, 1877; Keilin, 1924, 1944; Davies, 1927; Wigglesworth, 1938; Keister, 1947, 1948). We shall call the process by which gas replaces the tracheal liquid "tracheal filling."

It is frequently assumed that the gas which appears in the new tracheae of insects with open tracheal systems is atmospheric air which has entered through the spiracles. This assumption is supported by the fact that in some insects the tracheae do not fill with gas unless the spiracles are exposed to free air. In other species, however, the tracheae can fill with gas even when the larva or embryo is submerged in water or surrounded by amniotic fluid, and many aquatic insects with closed systems normally fill their tracheae without apparent contact with an external source of gaseous gas (for literature see Keilin, 1924, and Sikes and Wigglesworth, 1931). It thus appears that in different insects there are at least two distinct mechanisms of tracheal filling, differing as to the source of the gas.

A number of explanations of tracheal filling have been proposed.

Weismann claimed that growth of tracheae (increase in diameter and number) continues after a change in permeability of the lining prevents entry of further liquid. The original liquid then retreats into the finer branches where it is absorbed, and gas enters from the outside or diffuses in from the tissues to occupy the increased tracheal volume. Major objections to this idea are the facts that, at least in Sciara, the tracheae have the same diameter when originally laid down as when they fill, and that no new branches are added near the time of filling (Keister, 1948).

Stadtman-Averfeld (1923) suggested that the violent body movements at molting expel the liquid from the tracheae, after which air enters through the spiracles.

Tillyard (1916), from the equivocal observation that tracheae collapsed when dragonfly larvae were put in KOH, proposed that the gas in the tracheae was CO_2 which diffused in from the hemolymph and displaced the liquid. Keilin (1924) pointed out several difficulties with this hypothesis. An additional objection is that the hydrostatic pressure in the tracheal liquid would have to be less than in the body liquids in order for gas evolution to be confined to the tracheae.

Filling has been attributed to reduction in body hydrostatic pressure at hatching (Davies) or after eclosion (Fraenkel, 1935). However, such reduction would not bring about the disappearance of the tracheal liquid unless the pressure had previously opposed some other force (e.g., osmotic) tending to withdraw the liquid into the body. Furthermore, it would not necessarily lead to the concurrent appearance of gas in the tracheae except in insects with spiracles open to the air. In closed tracheal systems, reduction in hydrostatic pressure might indeed bring about a preferential evolution of gas in the tracheal liquid (in the unlikely event that the tracheal liquid was higher in dissolved gases than the other body liquids) but would not account for the disappearance of the liquid itself. In any case, the facts that some embryos fill their tracheal systems before hatching, and that many insect eggs apparently have a low internal pressure (Sikes and Wigglesworth), argue against the idea that hydrostatic pressure has any major role in tracheal filling.

From ingenious experiments on the osmotic control of gas movement in the "tracheoles" of mosquito larvae, Wigglesworth (1930) postulated that increased tissue osmotic pressure due to muscular activity at molting could explain tracheal filling. Such a mechanism might conceivably lead to the replacement of tracheal liquid by outside air in insects with open systems, if the tracheal wall were semipermeable and the tracheal liquid markedly hypotonic to the body liquids as Wigglesworth postulated. However, neither of these conditions has been shown directly and unequivocally to occur, and indeed the latter seems rather improbable in view of Keilin's (1924) and Wigglesworth's (1939) opinion that the tracheal liquid is molting fluid, and Keister's demonstration that it originates by direct cytoplasmic transformation.

Wigglesworth later (1938) discovered that mosquito larvae hatched and kept completely under water for some days do not fill during the same stadium, even when returned to contact with air. From this and other considerations he concluded that the essential factor in normal filling was "secretion" of gas by the cells bounding the tracheae (these presumably being inactivated by long hypoxia in the submersion experiments). Gas secretion by hypothetical specialized cells or protoplasm was also invoked by Sadones (1895), Winterstein (1912), von Frankenburg (1915) in connection with this hydrostatic pressure theory, Pause (1918), and Akehurst (1922). A major objection to this proposal is the lack of any direct cytological, physiological and physical evidence of how secretion of gas might occur.

Keilin (1924) suggested that sudden absorption of tracheal liquid by the surrounding tissues (due to "imbibition or to a chemical reaction") would allow gas to diffuse from the tissues into the vacated space, provided the trachea could withstand a pressure of about an atmosphere. Bult (1939) similarly proposed imbibition as the explanation of the movements of tracheolar liquid and gas which Wigglesworth had attributed to osmotic forces. However, in neither Keilin's account nor in Bult's elaborate hypothesis is there any concrete evidence of the existence or nature of imbibitional changes.

An important characteristic of tracheal filling which must be accounted for by any proposed explanation is its great speed. It seems evident that at least the growth hypothesis of Weismann does not meet this requirement, and it has not been demonstrated that any of the other postulated mechanisms do.

The above review indicates that tracheal filling is far from being satisfactorily explained, and that it has not always been recognized that a process which might account for the appearance of gas in the tracheae would not necessarily explain the disappearance of liquid, or vice versa. The present investigation was undertaken in an attempt to clarify some of the problems outlined above.

Material and Methods

Living larvae of the mycetophilid fly *Sciara coprophila* Lintner were used usually at the beginning of the fourth stadium. The anatomy and development of the tracheal system, the process of molting and methods for raising larvae of known age have been described previously (Keister, 1948). Exposure to gases was carried out in brass cylinders each 22 mm. in diameter, 8 mm. high and with a wall 1 mm. thick. Each chamber had lateral inlet and outlet tubes and was cemented by one rim to an ordinary microscope slide. High humidity was maintained by lining the chamber wall with wet filter paper, and by bubbling the incoming gas through water. The larva was placed ventral side up in a minute droplet of water on a coverglass which was then inverted on the greased top rim of the chamber. Larvae so mounted could be observed for many hours, and at all magnifications. The work was done at 25 ± 3° C.

For convenience in finding larvae in the proper stage, cultures were made up in petri dishes. Individuals about to molt are recognized by the fact that they stop locomoting and feeding and lie extended, usually with the anterior end raised, and make only slight and slow movements. Also, the tracheae are less distinct than in younger third instars due to the future fourth instar system enclosing them, and to the fact that usually much of the gas in the third instar system is replaced by liquid shortly before molting occurs. Further characteristic behavior usually involves: a period of axial back-and-forth rotation of the proventricular region of the gut; a period of slow, strong peristaltic waves of the body wall, alternately forward and backward; and finally, repeated bulging of the body just behind the head capsule. Soon thereafter the dorsal wall of the capsule and the cuticle just behind it split longitudinally, and the larva frees its anterior end and crawls forward out of the tubular exuviae, which are anchored to the substratum by viscid strands. Since the new tracheal system normally remains liquid-filled only for a short time after molting, larvae were mounted as quickly as possible after the splitting of the head capsule (usually within 1 minute). It was generally desirable to hasten the larva out of the cast by gentle prodding at the posterior end.

Observations

Normal tracheal filling

As described previously (Keister, 1948), visible gas is not present in the tracheae of newly-hatched (first stadium) Sciara larvae, and is found in only about one larva out of three even at the end of the stadium. When present, however, its extent is very constant. The actual filling process was never observed in spite of very numerous attempts. However, for reasons given in the earlier paper, it is considered unlikely that the gas enters by way of the posterior spiracles, which are the only pair present at this stage of development.

Second and third stadium larvae are less favorable for detailed study than fourth instars because of their smaller size. However, enough younger larvae were studied to show that tracheal filling after the first and second molts is essentially the same as described below.

Following the third molt, gas appears in 3 to 8 minutes and spreads throughout the (fourth instar) system in 1 to 2 minutes. The gas completely fills the principal trunks almost instantly and apparently at a rather uniform rate. The lateral branches and spiracular connectives do not begin to fill until after the filling of the main trunks is complete, and they fill more slowly. Gas extends to the ends of the finest branches ("tracheoles"). Filling is initiated and progresses without any visible change in the normal locomotion and activity of the larva.

In normal larvae with spiracles in contact with air, careful and repeated observations under high magnification showed that gas first appears at some single point within the principal trunks, usually in one of the first four body segments. Although the suddenness of filling usually made it impossible to be absolutely certain of the exact spot where filling began, in a number of conclusive instances it began at some point other than a spiracle, and in no instance was it seen to begin at a spiracle. Ordinarily the gas spreads progressively and continuously both forward and backward from the starting point, and fills one main longitudinal trunk completely before crossing over by either the anterior or posterior commissures or both to spread through the tracheae of the opposite side. However, under conditions where filling has been interrupted experimentally (see below), it occasionally restarts in a new liquid-filled region, rather than continuing from its original stopping point.

Since the entire new system is still liquid-filled just after molting is completed, the tracheal liquid does not escape with the molted tracheae. Similarly, since the gas can be seen progressing distally into the liquid-filled branches (which end blindly), the liquid does not leave through any of the spiracles. This is particularly convincing after the first or second molt when the new system has only one pair of developed spiracles (anterior), and the gas can be seen passing posteriorly into regions where there are no possible exits. Furthermore, in larvae filling under oil (see below) no escaping aqueous liquid was seen. Also, liquid was sometimes seen to disappear from sctions of tracheae (usually the closed loops of the large lateral trunks) when gas approached simultaneously from both directions as Wigglesworth has also observed. From the above considerations it follows that the liquid passes through the tracheal and tracheolar walls into the blood and tissues as the gas appears.

Tracheal filling in submerged larvae

Since the open tracheal system of Sciara can, and probably normally does, fill independently of the spiracles, it was of interest to ascertain whether or not submerged larvae can fill their tracheae with gas. Of 27 late third instars submerged in a half inch of aerated water, 21 succeeded in molting in the course of 2 days, although they were not able to free themselves completely from the old cuticles for lack of a relatively dry surface for attachment. The tracheae of these larvae became gas-filled except for occasional individual tracheoles or segmental units. Normally no gas passes from the old to the new tracheae; but even if it did under the abnormal circumstances of the test, the amount would be far too small to fill the fourth instar system. As a possible indicator of whether the gas comes from an internal or external source, the experiment was repeated with freshly-molted larvae (i.e., with completely liquid-filled tracheae) taking care to exclude all air-bubbles from the dishes. Twenty or more larvae were used in each of three liquids: ordinary water, freshly boiled water (the containers being filled to the top and covered), and a 6 mm. layer of mineral oil. In every instance filling began in approximately normal time and proceeded in the usual fashion. These experiments indicate that a visible external gas bubble is not necessary to initiate tracheal filling, in agreement with the previously described observations under high magnification. Furthermore, since the amounts of dissolved gas were probably quite different in the three liquids, the results suggest first that the gas which fills the tracheae of submerged larvae comes from some internal source, and second that filling is independent of O_2 tension over a wide range.

Tracheal filling in gases other than air

To investigate further the nature of the tracheal gas and the role of O_2 in filling, fresh ecdysiasts were exposed to commercial N_2, CO_2 and CO [1] in the gas chambers previously described.

Although there were considerable variations in the individual responses, the larvae were completely immobilized by a few minutes' exposure to any of the gases. In most instances gas appeared in portions of the main tracheae either in normal time or within 30 minutes and usually after the larvae were motionless. However, filling was not completed unless air was admitted to the chamber. Filling could be stopped and restarted repeatedly by alternating exposures to the tank gas and to air.

If the gases used were passed over hot copper gauze before admission to the gas chamber, filling was completely inhibited for an indefinite period (2 hours was the longest exposure tried). If the larva was returned directly to air after a period of complete anoxia not exceeding 75 minutes, filling usually was normal. Longer periods of complete anoxia often led to incomplete or delayed filling, and sometimes to complete and permanent inhibition. Permanently affected larvae died after a few hours, though they might, for a time, resume body movements, gut peristalsis and heartbeat. Filling was not visibly affected by pure medicinal O_2.

The quantitative relations between tracheal filling and O_2 tension, and differences between the specific effects of the individual gases, will be reported in detail in another communication, but for present purposes it may be said that, if given initially, mixtures of 0.3 per cent O_2 and 99.7 per cent CO_2, CO or N_2 suffice to permit gas to appear in the liquid-filled tracheae.

Effect of low temperature on tracheal filling

In freshly-molted larvae placed in small droplets of water on glass kept on melting ice, filling was indefinitely delayed (2¼ hours was the longest exposure tried). When returned to 25°, filling was normal but could be halted and restarted repeatedly by alternately chilling and rewarming the larva. During the exposure to 0° the larvae were practically motionless but responded markedly to mechanical stimulation.

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[1] No difference was observed between experiments done in the light and those done in the dark, using a deep red filter to examine the larvae.

Experiments with hydrostatic and osmotic pressure

Attempts were made to prevent filling by exerting pressure from a coverglass on larvae mounted on a slide either in water or in air. It was found impossible to prevent filling from starting even with the greatest pressure which could be applied without bursting the larva.

The larval cuticle of Sciara is somewhat permeable to water, and exposure to hypertonic solutions causes withdrawal of water from the body. Since the consequent lowering of body turgor might accelerate filling, if release of hydrostatic pressure were a factor in filling, freshly molted larvae were immersed in double Ringer's solution. Filling was normal.

DISCUSSION

From the foregoing experiments the following deductions can be made concerning some of the previous explanations of tracheal filling: (1) Tillyard's hypothesis necessitates a gradient between body and tracheal liquids of CO_2, a gas with high aqueous solubility and diffusion coefficient. This would be rather improbable even for larvae in air, but seems quite out of the question in Sciara larvae, in view of their demonstrated ability to fill in 99.7 per cent CO_2. (2) The fact that filling does not occur in larvae completely relaxed by anoxia argues against mechanisms involving a fall in hydrostatic pressure, as do the experiments with coverglass pressure and hypertonic solution. Conversely, the fact that filling can occur in larvae motionless in 99.7 per cent CO_2 runs counter to Wigglesworth's claim that CO_2-narcosis *per se* inhibits filling, and to Standtman-Averfeld's activity theory. Muscular activity as a factor also seems to be ruled out by the lack of filling after the body contractions induced in chilled Sciara larvae by prodding. (3) The inhibition of filling by anoxia argues against both osmotic pressure and imbibition as prime factors in filling, since both are claimed to be enhanced by anoxic catabolism. (4) Weismann and Keilin believed that the tracheoles were the site of absorption of tracheal liquid, but our findings that liquid can leave through trunk walls and that the trunks fill completely before gas enters any of the side branches indicate that the filling mechanism also operates through the main tracheae. This is supported also by the apparently rather uniform rate of filling in the main trunks (where a deceleration would be expected if the liquid were leaving via the side branches). An additional puzzle is that most reports agree that the largest trunks, which have the highest ratio of liquid content to surface area, fill in a matter of seconds; whereas, the fine, thin-walled branches, where it seems that imbibition, osmosis, etc. should be more effective, fill much more slowly. (5) The fact that the tracheal filling of the first instar does not occur for a day or more after hatching (if at all), whereas tissue osmotic pressure might be expected to be highest during the struggles of the embryo at hatching, militates against the osmotic theory. (6) From the fact that the Tenebrio embryo can fill its tracheae either with outside air or with gas derived from tissue fluids, Sikes and Wigglesworth concluded that there is no essential difference between filling in closed and in open systems. Although this conclusion does not appear justified logically, our finding that Sciara, which has an open tracheal system, normally may fill its tracheae with gas from some internal source, suggests that other insects (e.g., Tenebrio) likewise normally may fill as if they had closed systems in spite of opportunity to take in outside gas. (7) Our demonstrations that (at ordinary temperatures) O_2 is an absolute prerequisite for tracheal filling, and that filling may be delayed by O_2-poor conditions, offer a possible explanation for the observations of Sikes and Wigglesworth that the Lucilia embryo will fill under water, but only if near the surface; of von Frankenberg that Corethra will not fill its tracheae if kept in boiled water; and of Tillyard that dragonfly larvae in O_2-poor water filled slowly. (The first observation was not elaborated by the authors; the second was attributed to insufficient dissolved gas in the surrounding water to serve for filling the vesicles; and the third to "weakening" of the larvae.) (8) The strict O_2-dependence of the initial filling of tracheae makes it questionable whether the "metabolic" movements of tracheolar liquid and gas studied by Bult are brought about by the same mechanism (though both processes are CO-insensitive) since Bult's postulated imbibitional mechanism is apparently anaerobic.

The net result of the above discussion is to eliminate from serious consideration as prime factors in tracheal filling all proposed mechanisms except those postulating "secretion" of gas. As previously stated, however, this concept is so vague that no critical consideration of it is possible at present. Insofar as "secretion" is a metabolic phenomenon, it is compatible with our finding that tracheal filling is absolutely dependent on O_2 and can be indefinitely inhibited by low temperature. However, we have no evidence as to what metabolic change might be involved, except that it is unlikely to be mediated by the cytochrome system. Numerous other aerobic processes (not all necessarily metabolic) are conceivable. Tillyard for example regarded O_2 as essential for a metabolic process by which gases dissolved in the external medium were transferred into the body and liberated in the tracheae.

It might, of course, be assumed that the *initiation* of filling and the actual filling process depend on quite different mechanisms. One could imagine for example that the reaction which requires O_2, and which might be metabolic, simply pulls a trigger which sets off a physical process resulting in tracheal filling. A number of possible mechanisms will be dealt with in another communication, but the evidence already available permits several deductions. Keister (1948) reported that scraps of air-filled third instar tracheae sometimes break off and are left within the new system at molting. The fact that filling will not start, or if previously started will not continue, in such larvae in the absence of oxygen or near 0° C. shows that filling does not progress automatically once gas bubbles are present in the tracheal liquid. The further fact that filling, when it occurs, does not necessarily begin in regions containing tracheal scraps, shows that preformed gas bubbles are not a prerequisite for the initiation of filling. The same conclusions follow from the fact that after filling has been interrupted experimentally it sometimes resumes in a new (liquid-filled) region rather than continuing from its original stopping point. Finally, since filling will occur in gases ranging from tank O_2 to practically pure CO_2, CO or N_2 it is very unlikely that either the initiation or the progress of filling depends on the attainment of any critical ratio between gas concentrations, or upon the presence of any specific gas (except O_2).

SUMMARY

(1) After each molt the new tracheal system of a Sciara larva in air normally remains filled with liquid for 3 to 8 minutes. It then fills spontaneously, rapidly

and completely with gas, beginning at some point in a main trunk. The gas comes from some internal source.

(2) Sciara larvae can molt under aerated water, and can fill their tracheal systems with gas while completely submerged in aerated or boiled water, or mineral oil.

(3) Tracheal filling can occur if the larva is in a mixture of 99.7 per cent CO_2, CO or N_2 with 0.3 per cent O_2, but not in complete absence of O_2.

(4) Tracheal filling is indefinitely inhibited near 0° C.

(5) Filling may be stopped and restarted repeatedly by alternating exposures to anoxic gas and air, or to low and room temperatures.

(6) Initiation and progress of tracheal filling are apparently independent of body movements, body hydrostatic pressure, and critical gas ratios.

(7) It is suggested tentatively that tracheal filling involves a metabolic process.

(8) A review and critique of previously proposed mechanisms of tracheal filling is presented.

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AN ABBREVIATED CONJUGATION PROCESS IN PARAMECIUM TRICHIUM

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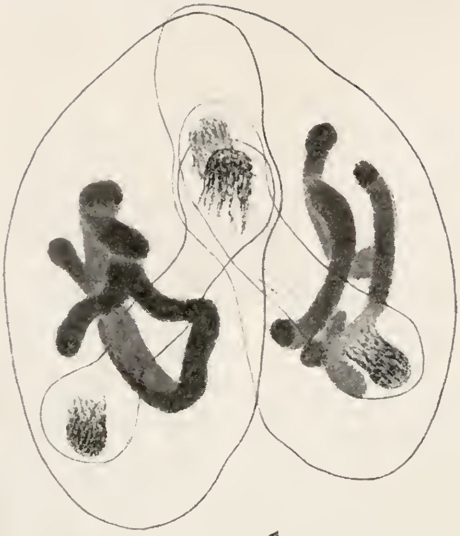
The remarkably constant, well-ordered, complex series of nuclear processes which are characteristic of ciliated protozoa during conjugation has been established by a host of cytological investigators. The almost monotonous regularity of the maneuvers in many species of ciliates during conjugation has led to a fairly stereotyped concept of the events of conjugation in ciliates generally; three pregamic divisions producing the pronuclei (with degeneration of nuclei after the first and/or second divisions), interchange of gametic nuclei, fertilization, and the reorganization of a new nuclear complex from the synkaryon after a characteristic number of divisions. The invariability of this "standard" process was called into question recently by a number of investigators including the author (Diller, 1936), who suggested that conjugation might not always involve an exchange of pronuclei and reciprocal fertilization, but fusion of pronuclei arising in the same member of the pair (autogamy). Both cytological (Wichterman, 1940; Chen, 1946; and Diller, 1948) and genetic (Sonneborn, 1947) studies have subsequently demonstrated the reality of autogamy in conjugation. Moreover, genetic effects due to cytoplasmic interchange during conjugation have been claimed by Sonneborn (1943, 1945) and Dippell (1948). Another event in the classical picture of conjugation—the puzzling third pregamic division—has now been shown to be not indispensable. In certain races of *P. trichium* (Diller, 1948) the conjugants may omit the third division and proceed with either reciprocal fertilization, autogamy ("cytogamy" of Wichterman) or parthenogenetic development of gametic nuclei.

In view of the great versatility of nuclear behavior shown by *P. trichium* during conjugation (Diller, 1948) and the large favorable micronuclei which this species possesses, it would seem to be of interest to describe a further variation from the "standard" conjugation behavior. In this heretofore undescribed process certain stages are eliminated and the micronuclei proceed directly and without degeneration of their products to establish a new nuclear apparatus.

SOURCE OF MATERIAL AND TECHNIQUES

All the material on which the present study was made, was derived from a pond collection kindly furnished by Dr. Hannah Croasdale of the Department of Zoology, Dartmouth College. The collection was taken on September 20, 1946, from a pond on Dr. Carleton's grounds in Hanover, N. H. Some of this material was introduced into hay infusion on September 23, 1946, and on the next day about fifteen pure-line mass cultures, each descended from a single animal, were isolated from this culture. Several small mass cultures were also established at this time. No

PLATE I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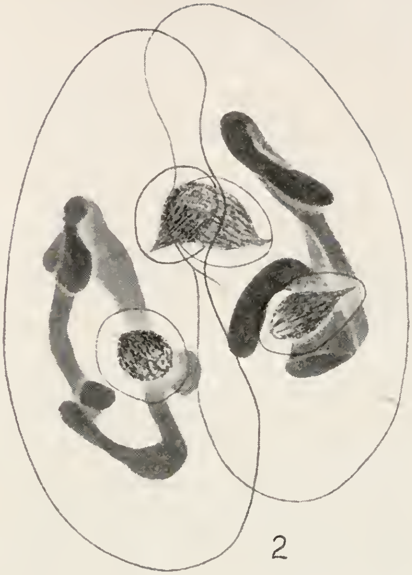
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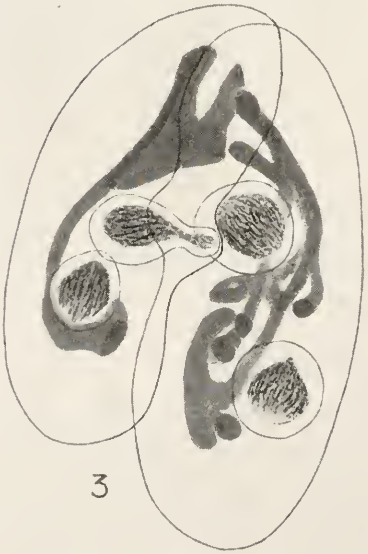
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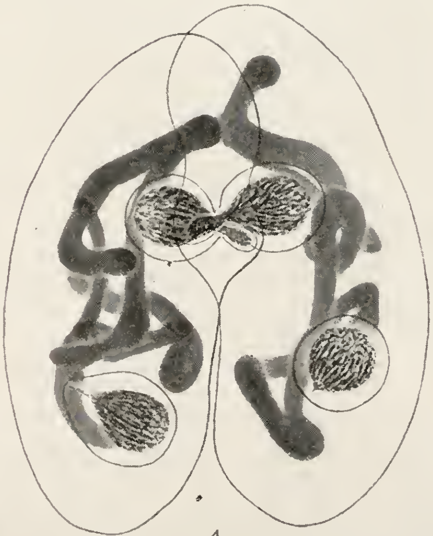
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significant differences in cytological behavior between the various lines were noticed, although most of them were examined from time to time and the selection of material to be studied was more or less random. Very shortly after their establishment, conjugation occurred in many of the cultures; for instance, in isolation culture No. 10, conjugation was in progress by October 3.

In most of the lines conjugation occurred in at least small numbers, at all times. The cultures were maintained on hay and malted milk, boiled in pond water. Several of them (isolation cultures Nos. 5 and 6) were mixed. Although there were small numbers of conjugants in each culture at the time of admixture, the combination resulted in a rather heavy incidence of conjugation so that it would seem as if these two cultures may have been opposite mating types. The cultures were maintained until June, 1947, when they were abandoned. Toward the end of their life span the cultures showed a more conventional behavior and finally did not conjugate at all. Temperatures in the laboratory became rather high and this may have been responsible for the decline of the cultures.

All the observations reported in this paper were made on killed and stained material. The animals were pipetted from the cultures into a centrifuge tube, concentrated, allowed to stand for a few minutes, and then fixed in Perenyi's fluid. They were sometimes subsequently treated with Schaudinn's fluid, and stained in acetic orcein or in Grenacher's alcoholic borax carmine. Both stains gave very good results. Usually fast green or indulin were used as counterstains. All the technique was carried out in the centrifuge tube and the animals mounted on slides in diaphane or clarite.

EXPLANATION OF PLATES

These are camera lucida drawings of stained whole animals from six isolation cultures started Sept. 24, 1946, and a small mass culture started Sept. 28, 1946, all derived from Carleton Pond, Hanover, New Hampshire. Magnification about 1200 times. All the figures illustrate *Paramecium trichium* during "abbreviated" conjugation. The animals were fixed in Perenyi's fluid and stained with Grenacher's alcoholic borax carmine or acetic orcein and counterstained with indulin or fast green. The specimens shown in Figures 1, 2 and 9 are representatives of small mass culture A; Figure 3, isolation No. 15; Figures 4 and 5, isolation No. 10; Figures 6, 7, 10, 11 and 16, isolation No. 14; Figure 8, isolation No. 6; Figures 12 and 15, isolation No. 9; Figures 13 and 14, isolation No. 17. Only the nuclear structures have been drawn. In some of the figures, particularly the later stages, old macronuclear fragments lying on top of the structures which were intended to be illustrated were omitted for the sake of clarity.

PLATE I

EXPLANATION OF FIGURES

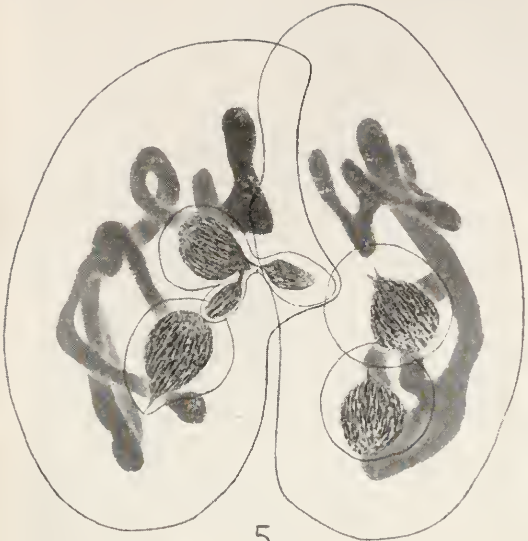
FIGURE 1. Telophase of first maturation division in a culture engaging in "abbreviated" conjugation. The macronuclear skeins (simple) seem to develop earlier than they do during "standard" conjugation. A daughter nucleus is found in the paroral cone region of each conjugant.

FIGURE 2. Two nuclei resulting from the first division. Each has a tail indicative of recent separation.

FIGURE 3. Two nuclei in each conjugant. Twisted chromosomes and a knob on the nucleus in the paroral cone region of the left conjugant are suggestive of its impending passage. Since the corresponding nucleus in the right conjugant is not in a similar condition, this pair suggests a one-way passage.

FIGURE. 4. "Migratory" nuclei produced after the first division. A nipple-like process on each extending toward external boundary of cone. Suggestive of reciprocal transfer.

Plate II



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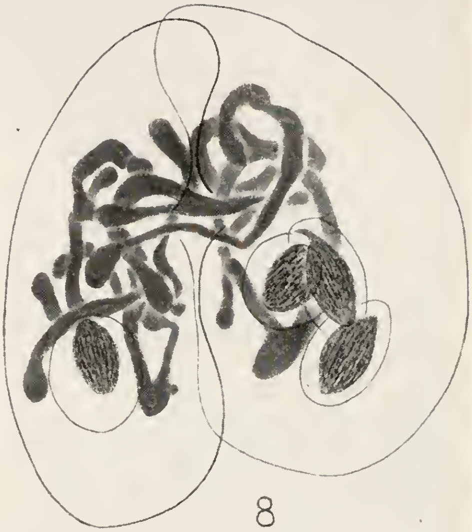

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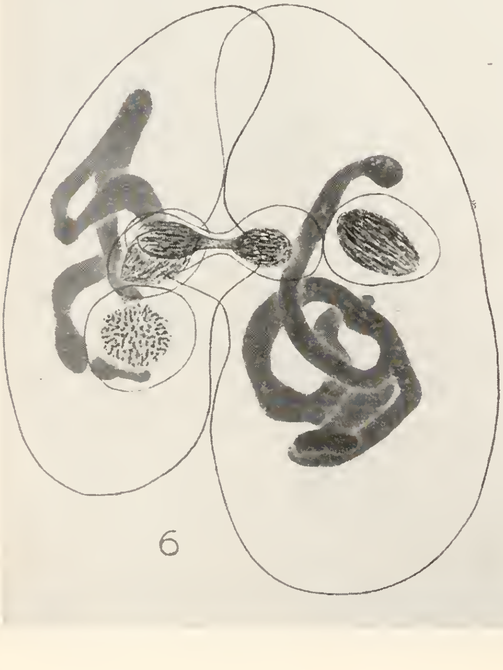

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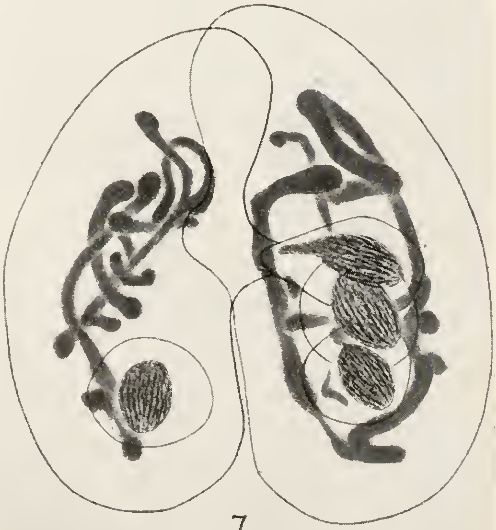

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Observations

Although no observations were made on the length of time that the members of the pair remain attached in abbreviated conjugation, it is my distinct impression that the time is much less than for the conventional method. It is probable that the first division of the micronucleus consumes less time than ordinarily is the case. The macronucleus in abbreviated conjugation seems to be somewhat precocious in its skein formation. By the time of the telophase of the first division, a simple macronuclear skein (Fig. 1) has formed. One sister chromosome group of each spindle is likely to be found in, or near, the paroral cone. The daughter nuclei, immediately after separation, frequently have "tails" on them (Fig. 2). This is true also for the corresponding post-telophase stage of the other divisions. Normally, the first division is not followed by degeneration. I have seen only one or two pairs, in abbreviated conjugation, in which degeneration of nuclei was evident. This is rather unusual because in conventional conjugation, as well as in the type in which only the third pregamic division is omitted, degenerating nuclei after the first and second divisions are the rule.

A reorganization of the two nuclei in each conjugant leads to a premetaphase condition (Fig. 3 and others). A knob-like, nipple-like, or handle-like process (Figs. 3, 4, 5, 6, 7, 8 and 10) is formed on one (Fig. 3) or two (Fig. 4) of the nuclei. This modification marks the nucleus as a potential migratory gametic nucleus. Undoubtedly, it is reflective of cytoplasmic stresses, pressures and/or currents in the cone regions. The narrow pointed process may be directed toward the cell boundary or toward the interior of the cell. The chromosome threads are frequently arranged in a spiral fashion, suggesting a twisting influence on the nucleus. In Figure 3, the presence of a single nucleus in the paroral cone of the left conjugant, with a terminal knob, and the absence of a similar structure in the right conjugant suggest an imminent one-way passage. Frequently, the macronucleus of one conjugant is a little more advanced in skein formation than is the other (Fig. 3). In contrast, two such migratory nuclei (Fig. 4) may be present, indicating an approaching reciprocal transfer.

Occasionally the pinching effect appears to be so severe as to cause a disruption of the migratory nucleus into several parts. Such an instance is represented in Figure 5. It is conceivable that this process may be the means whereby small accessory nuclei arise, by a purely amitotic mechanism. This possibility will be considered later in connection with subsequent stages. It is probable that most of these constricted nuclei would recondense and adjust to the normal condition after

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Plate II

Explanation of Figures

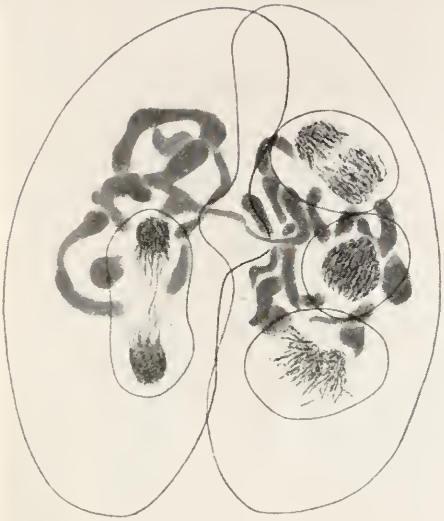
Figure 5. The "migratory" nucleus of the left conjugant pinching off two small portions, each connected to the larger section. One of the small accessory nuclei is lying in the cone.

Figure 6. Macronuclei in simple skeins. No degenerating micronuclei. Migratory nucleus, constricted in the middle, passing through the cone from the right conjugant to the left. One-way passage.

Figure 7. One-way passage of migratory nucleus, after first division, from the left conjugant to the right one. Tail of migratory nucleus still in tip of cone.

Figure 8. One-way passage of a migratory nucleus. Interchange of rather coarse macronuclear strands in both directions.

Plate III



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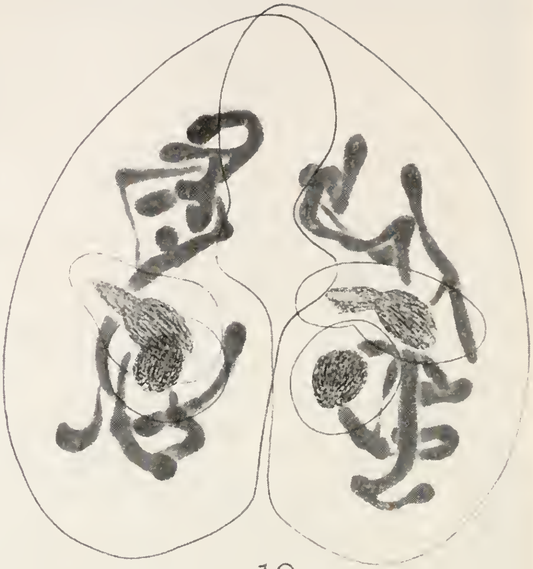

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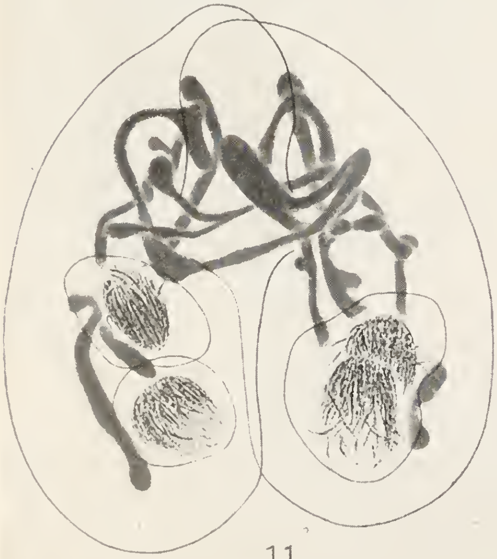

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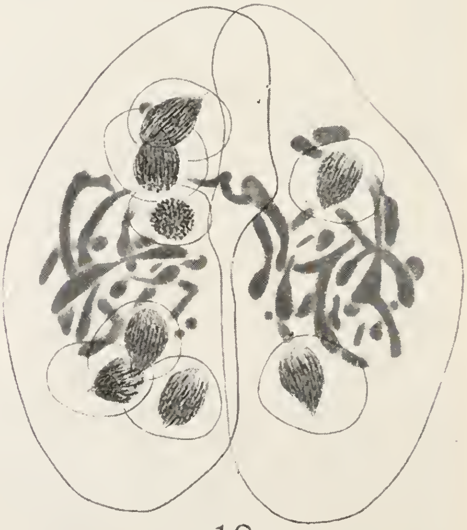

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the temporary stresses had been relieved. I have seen one other instance, not figured in this paper, which could produce a similar result. One member of the pair had two normal nuclei from the first division. The other had a tripolar telophase of the first division. One of the three sister chromosome groups was smaller than the other two.

At the time when the migratory nuclei are actually passing through the paroral cones they often show an equatorial attenuation. Such a dumbbell effect is shown in Figure 6. The migratory nucleus is passing into the small left conjugant. There is no indication of nuclear passage in the reverse direction. The macronuclear skeins are still relatively simple and coarse. In this case there is cytoplasmic continuity at one level only. Figure 7 illustrates a slightly later stage of one-way passage of a migratory nucleus after the first division. Its tail is still in the cone region. Probably in this pair there is cytoplasmic continuity between the conjugants at two levels.

It is difficult to ascertain the frequency of the occurrence of the different modes of behavior of the nuclei, after the first division, in the Carleton Pond stock of *P. trichium.* One-way passage of a nucleus, leading to the spectacular unbalanced condition of three nuclei in one member and one nucleus in the other conjugant—a situation which first attracted the author's attention to this process—is by no means a rarity in these stocks. Two-way passage (interchange), as suggested by Figures 4, 10 and others, is also quite common. In the event of an original heteroploidy of the micronuclei of the two conjugants, it is possible to determine at a later stage whether interchange had occurred. A third alternative is evident: the failure of nuclei in both conjugants to migrate and their development in the same conjugant in which they arose. This possibility is, in the author's opinion, a valid one, but seems to be more rare than the other two. Apparently, breakdown of the tips of the paroral cones, cytoplasmic currents, and/or internal pressures at the proper stage are the factors which determine the movements of nuclei at this time. The nature of these forces is entirely conjectural but it is of interest to note that they may be unequal in the two members of the pair.

Shortly after the passage of the micronuclei, or their non-passage, strands of the macronuclear skein may, or may not, become stretched across the cone regions from one conjugant to the other in much the same fashion as in unabbreviated conjugation (Diller, 1948). Passage of the macronuclear skein may be unidirectional

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PLATE III

EXPLANATION OF FIGURES

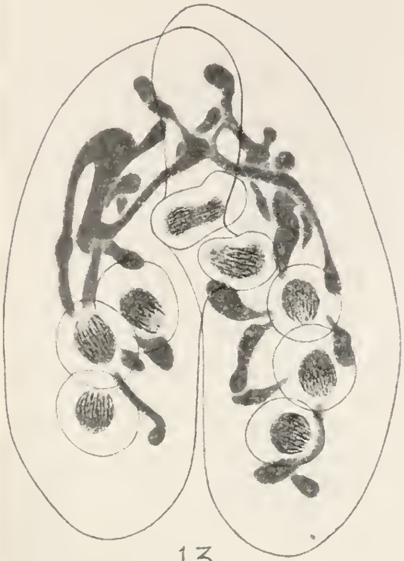
FIGURE 9. One micronuclear figure in left conjugant; three dividing nuclei in right conjugant. Second division. Macronuclear strand passing from left conjugant into right. All the nuclei are in slightly different mitotic stages.

FIGURE 10. Two gametic nuclei in each conjugant. Either cross-fertilization, autogamous fertilization, or parthenogenesis, is imminent. Uncertain whether there is cytoplasmic continuity in oral cone regions. The "migratory" nuclei are retaining the constrictions characteristic of the migratory condition.

FIGURE 11. Synkaryon formation in right conjugant. Two separate nuclei in left conjugant. In the latter, either nuclear fusion is delayed or the gametic nuclei are going to develop parthenogenetically. Macronuclear interchange in both directions.

FIGURE 12. Six nuclei in left conjugant; two in right conjugant. Macronuclei in short complicated strands.

PLATE IV



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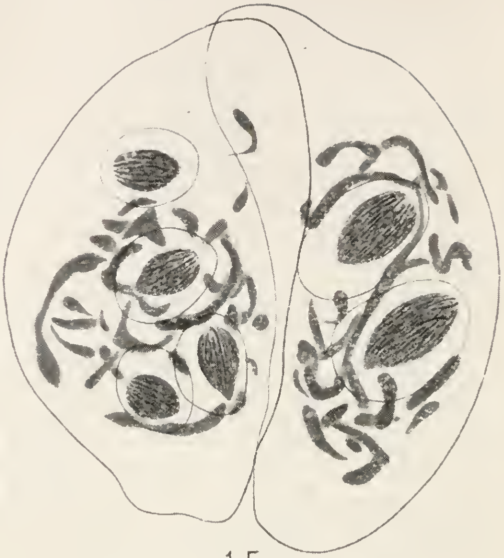

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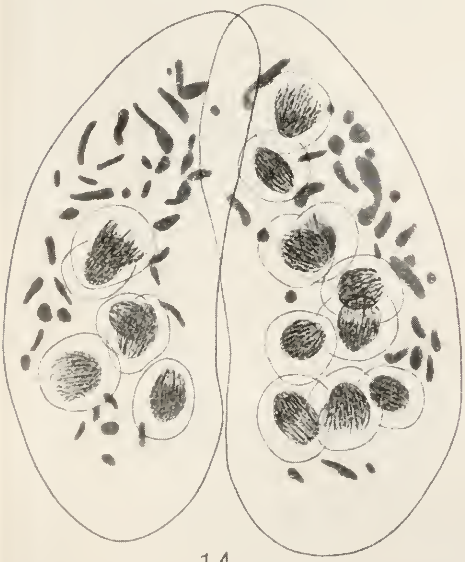

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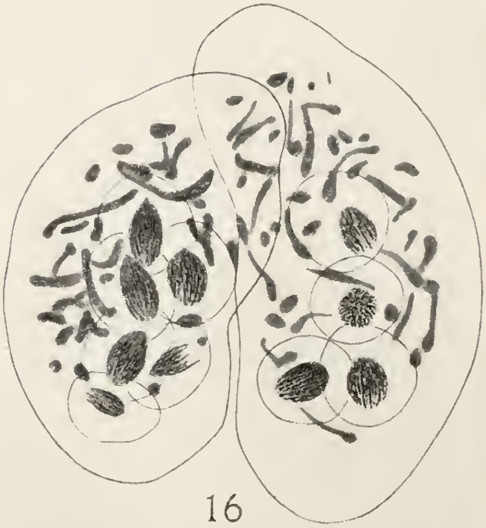

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(Fig. 9) or may extend in both directions (Figs. 8 and 11). Macronuclear exchange was very frequent in the Carleton stock. In case it is unidirectional, the strands can pass either from the uninucleate conjugant into the trinucleate member, as in Figure 9, along the path which the single migratory nucleus took, or in the reverse direction. The direction of macronuclear movement seems not to be directly correlated with the direction of micronuclear movement. Exchange of macronuclear material apparently marks the end of interconjugant micronuclear movement.

As remarked above, there is normally no degeneration of nuclei at this stage, or any other stage, in abbreviated conjugation. One can detect several alternative modes of behavior of the nuclei from this point, keeping in mind the possibilities that the nuclei of one member of the pair may be behaving differently from those of the other member, and even that the nuclei in the same conjugant may be diverse in their activities. First, fertilization (synkaryon formation) may occur. Such a condition is shown in the right conjugant of Figure 11 and such was probably the ancestry of the two nuclei in the right member of Figure 15. Depending on whether interchange had occurred, cross-fertilization or self-fertilization would be accomplished. Second, parthenogenetic development of the nuclei may take place. This seems to be the most frequent type of activity in abbreviated conjugation. Third, combinations of fertilization and parthogenetic development may be adopted. Although the critical stages are rare, it is possible by reason of size differences to reconstruct previous history.

Figure 9 illustrates the micronuclear activity of the second division. The conjugant on the left contains a late anaphase micronucleus, while that on the right has three dividing nuclei in slightly different mitotic stages. It is a little unusual for the micronuclei to show such asynchrony. Probably the two anaphase nuclei, one in each conjugant, are sisters. It seems likely that all of these nuclei are developing without fertilization (parthenogenetically).

It is difficult to be sure about the exact history and the immediate fate of the nuclei of Figure 10. The "tailed" nuclei may have been interchanged, or not, and may be on the point of fusion with the stationary nuclei. Otherwise, parthenogenetic development would be expected to follow.

Figure 11 shows synkaryon formation in the right conjugant and two separate

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PLATE IV

EXPLANATION OF FIGURES

FIGURE 13. Unusual interchange at the end of the second division, with no degenerating nuclei apparent. The nucleus at the top is pressing against the cone moving toward the left, while the nucleus directly below it is part-way through the cone, passing into the right conjugant.

FIGURE 14. Six nuclei in left conjugant. Ten nuclei in right conjugant. Presumably, this condition arose from a one-way passage of a gametic nucleus at the stage represented in Figure 13. Old macronuclei represented by closely packed short rods and spheres (many omitted).

FIGURE 15. Four nuclei in left conjugant. Two very large nuclei in right conjugant. Probably parthenogenesis has occurred in the left conjugant while synkaryon formation has taken place in the right member.

FIGURE 16. Four nuclei in right conjugant. Four large nuclei and two small ones in the left conjugant. The latter may have arisen from pinched-off parts of nuclei after the first division, as suggested by Figures 3 and 5. (They may have originated in the right conjugant).

nuclei in the left conjugant. In the latter, either nuclear fusion is delayed, or the gametic nuclei are going to develop parthenogenetically. The latter alternative seems to me the more probable, since my observations suggest that very little time elapses before nuclear fusion is completed.

Occasionally I have found that one or both of the conjugants at later stages possess nuclei of different sizes. Aside from the explanation of original heteroploidy of the conjugants, this can best be interpreted by assuming that the larger nuclei have arisen from synkarya, while the smaller ones have developed parthenogenetically. Assuming that the number of nuclear generations is the same in both conjugants, synkaryon formation in one and not the other will result in different numbers of nuclear products in the two members at later stages (cf. Fig. 15). Although this is not the only explanation, I believe that asymmetric synkaryon formation is a valid one. One wonders whether an extra postzygotic division is required for final reorganization since there has been a reduction in nuclear number in the conjugant which produced a synkaryon. Another device for bringing about unequal nuclear numbers in the two conjugants is for the mitotic stages to become slightly out of step with each other. I believe this to be a real, but rather rare, happening. However, in the uninucleate-trinucleate pairs the single nucleus seems often to be ahead of the three others (Fig. 9).

Figure 12 shows completion of the second division in a pair in which there has been one-way transfer. By this time the macronucleus has usually fragmented into complicated short strands and rodlets and no longer can be traced to the opposite cell. Apparently the paroral cone intercommunications heal over at this time. The conjugants separate after this stage or during the next (third) division.

Migration of nuclei in abbreviated conjugation is not completely restricted to the time immediately after the first division. Very rarely, interchange can occur after the second division. Two such cases are shown in Figures 13 and 14. In the former, each conjugant has four nuclei, one of which is located in the paroral cone and is projecting into the other animal, apparently on the verge of effecting interchange. A one-way transfer of this type would result in five nuclei in one conjugant and three in the other. At the conclusion of the third division of such a hypothetical case, six and ten nuclei, respectively, would be found in the conjugants. That is apparently the explanation of the asymmetric condition of the pair illustrated in Figure 14.

A rather unusual and interesting asymmetrical case is illustrated in Figure 16. Four nuclei, following the second division, are present in each conjugant. In addition, there are two small nuclei in the left conjugant. These may have arisen as "buds" pinched off the nucleus at the time of the first division, as suggested earlier, which have persisted through division. (See Fig. 5.) They seem perfectly viable and normal.

The events subsequent to the third division, when the animals separate, have offered no special points of interest. Presumably reorganization, anlagen formation, and disappearance of the old macronucleus are similar to the standard processes characteristic of *P. trichium* (Diller, 1948), although I have made no particular effort in these studies to work out the post-conjugant stages. Regularly, the mature exconjugants would be expected to have four macronuclear and one micronuclear anlagen.

Discussion

The abbreviated conjugation process in the Carleton race of *P. trichium*, reported in this paper, accomplishes the ends of nuclear reorganization in a remarkably simple and direct manner without wastage of micronuclei and without unnecessary stages. However, it is so unorthodox and so divergent from the conjugation pattern of other ciliates, and even of other races of the same species, as to pose problems about its general significance, and, in fact, about the meaning of certain phases of the conjugation process as a whole. The standard or conventional scheme of micronuclear activity in the ciliates involves three pregamic divisions (two have been reported in a certain race of *P. trichium*, Diller, 1948), and a variable number of postzygotic divisions which reconstitute the definitive nuclear complex. The term "postzygotic divisions" is here extended to include parthenogenetic divisions or generations as well as those of fertilization nuclei. It is borne in mind, of course, that variation in numbers of macronuclear and micronuclear anlagen is common but is fairly constant for a given species. In *P. trichium*, in the standard process, there are two or three pregamic divisions and three postzygotic divisions. In abbreviated conjugation three divisions, simply, are required to complete the process. (Possibly an extra division is appended in case synkaryon formation is involved.) Similar numbers of final nuclear products arise in both processes. The failure of nuclei to degenerate in the abbreviated process, generally, accounts for the end products being the same in number as in standard conjugation. The question then arises as to the homology of the nuclear generations in abbreviated conjugation with those in the standard process. In both, the first division shows a characteristic polarized (not crescentic) prophase stage. This may well be indicative of a maturation or a reductional process and is followed, in the standard conjugation, by one or two other divisions before fertilization or parthenogenetic development. However, in abbreviated conjugation there is no further division before nuclear exchange and fertilization (or parthenogenesis) are accomplished. If one were to assign the exchange period as a central reference point in both processes, then one can consider the first division in abbreviated conjugation as a maturation division and the second and third divisions as being homologous with the postzygotic divisions of the standard process. If this interpretation is valid, what can be inferred about the chromosomal cycle in abbreviated conjugation? A comparable problem was raised before (Diller, 1948) in connection with the omission of the third division in certain races of *P. trichium* and the parthenogenetic development of reduced nuclei; it was concluded that under these circumstances each conjugation would be expected to result in a progressive diminution of chromosome number. Unfortunately, direct observation of chromosome numbers in the various generations is very difficult, if not impossible, to make, and even estimates of nuclear size are not very satisfactory in spite of the large and comparatively favorable micronuclei of *P. trichium*. It has been considered axiomatic that two maturation divisions are necessary to bring about chromosomal segregation and reduction in mature gametes. This is undeniably accomplished in the standard conjugation process, even when the third pregamic division is omitted, but is doubtful in abbreviated conjugation.

Two possibilities suggest themselves. First, that reduction is completed in the later divisions and that the final nuclei are haploid, unless fertilization occurs. In the latter eventuality, the awkward situation of the occurrence of a maturation division before fertilization and another after fertilization would exist. A second, and more probable, speculation is that the gametic nuclei are not reduced but diploid, and the nuclei arising by parthenogenesis would remain diploid while those derived from synkaryon formation would be tetraploid (cf. Fig. 15). Although a good deal of heteroploidy was evident in these cultures, hypoploidy was not nearly as extreme nor as conspicuous as in certain other stocks which I have been studying.

In correlation with the shortened morphological manifestations, it would be interesting to know how the time relationships of abbreviated conjugation compare with those of the standard process. I have the impression that abbreviated conjugation takes a shorter time than the standard process, but no positive evidence on this point. Unfortunately, the cultures were discarded before this information was obtained, in fact, before it was realized that abbreviated conjugation was happening; and I have not been able to secure any more stocks from the Carleton Pond, although several collections were made. The causes of the induction of abbreviated vs. standard conjugation are also entirely unknown at present. It seems to be not entirely a racial or genetic effect, since there were some instances of standard conjugation in certain of the Carleton Pond stocks.

I know of no other conjugation study in ciliates in which nuclear transfer has been observed at the end of the first division. The mechanism of conjugation activity has apparently been accelerated to bring about nuclear passage two generations ahead of the time usually required: the tips of the paroral cones have broken down and the macronuclear skein is far advanced. The latter seems to be precocious and attuned to the prospective activity of the micronuclei. Transfer of the micronuclei may be unidirectional, resulting in the asymmetrical condition of one nucleus in one member and three in the recipient, or reciprocal (interchange), or, probably, there can be non-passage. Such a selection suggests a chance determination. A pinching or constriction of the "migratory" nucleus before and during passage may be extreme —so severe as to cause a complete separation of fragments from the nuclei. These may persist and continue an apparently independent existence. (One hesitates to apply the terms "migratory" and "stationary" nuclei to the products of the first division, with the implication that these are reduced nuclei and that they invariably are involved in interchange.)

As in other accounts of conjugation in *P. trichium* (Diller, 1948), macronuclear passage may occur in abbreviated conjugation, after micronuclear migration. The macronuclear exchange may be either reciprocal, unidirectional, or, probably, omitted. In case of unidirectional micronuclear passage, macronuclear exchange is not necessarily along the same path, i.e., from the conjugant with one nucleus into the one with three nuclei, but may be in the opposite direction from the trinucleate to the uninucleate conjugant.

Also, as in other processes of conjugation in *P. trichium,* the subsequent micronuclear activities may be variable: fertilization (cross-fertilization or autogamy), parthenogenetic development, or combinations of fertilization and parthenogenesis in the two members of a pair or even, probably, in the same member of the pair. The versatility, lability and variability of micronuclear activity in *P. trichium* should be susceptible to experimental attack and analysis.

SUMMARY

1. A process of "abbreviated" conjugation occurs in one race of *P. trichium* in which the number of micronuclear divisions is reduced to three (or possibly four) from the "standard" pattern of five or six.

2. There may be exchange of micronuclei at the conclusion of the first division. Frequently, unidirectional passage of a gametic nucleus occurs at this time so that an asymmetry results in the two conjugants, one of them having three micronuclei and the other conjugant one micronucleus.

3. The products of the first division proceed, directly, to reconstitute the new nuclear apparatus. This they do by synkaryon formation, parthenogenetic development or a combination of the two, usually dividing twice. There is no degeneration of nuclei between divisions.

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ADDENDA

Seminar, July 12, 1949, Marine Biological Laboratories

X-ray mutations and fecundity of Mormoniella. Marion E. Kayhart and P. W. Whiting.

Females were treated (4000–8000 r) and mated to untreated males. Lowest dose given is, by analogy with Habrobracon, well above lethal for metaphase eggs. Therefore all offspring must be produced from prophase or preprophase. Fecundity tests show decrease in offspring with increasing dose given to adult females—(1173/10) 117.30 per untreated female, (229/27) 8.48 per 4000 r treated, (91/40) 2.27 per 5000 r, (199/88) 2.26 per 6000 r, (105/86) 1.22 per 7000 r, (69/83) 0.83 per 8000 r. Treatment (4000 r) of middle-aged pupae is more effective—(26/40) 0.65 offspring per female—than of young pupae—(113/50) 2.26—, old pupae—(445/80) 5.56—, or adults. Treatment (6000 r) of middle-aged pupae is more effective—(36/40) 0.90 per female—than of old—(88/40) 2.20—or adults. This sensitivity of eggs of middle-aged pupae is probably due to injury to nurse and follicle cells, as well as to oocytes.

Among 1262 sons of treated mothers were five independently occurring eye color "mutants"—scarlet-2 (found dead), scarlet-3 (overetherized), pinkish (sterile), oyster (proved *oy*), tomato, *to* (fertile). Among 387 daughters given breeding test, in general producing over 30 sons each, 16 proved heterozygous for mutant traits—eye colors: oyster, *oy* (fertile), scarlet, *st* (fertile), vermilion, *vm* (fertile) (4 + : 5 mutants), vermilion (proved *vm*), garnet, *ga* (fertile) (58 + : 6 mutants), scarlet-4 (fertile) (14 + : 19 mutants, scarlet-5 (fertile) (50 + : 58 mutants), light scarlet (28 + : 9 mutants found dead); body colors: blue-1 (19 + : 29 mutants found dead), blue-2 (sterile) (1 + : 3 mutants), blue-black (sterile) (124 + : 57 mutants), greenish-blue (sterile) (33 + : 23 mutants), purple (sterile) (15 + : 12 mutants); wings: shredded (sterile) (37 + : 20 mutants); legs: short-1 (sterile) and short-2 (sterile). No mutations have been found among many thousands examined from untreated stock. The 22 from the treated were distributed irrespective of dose or of age at time of treatment.

Seminar, August 16, 1949, Marine Biological Laboratories

The Development of Menidia-fundulus Hybrids. James M. Moulton.

Menidia beryllina beryllina ♂ × *Fundulus heteroclitus* ♀ hybrids survive until the hatching of controls, as reported and briefly described by Clark and Moulton (*Copeia,* 1949, No. 2), but have thus far failed to hatch. The reciprocal cross advances only to the yolk-plug stage. Both crosses of the other *Menidia* race found in the Woods Hole region, *Menidia menidia notata,* with *Fundulus heteroclitus* survive only to the yolk-plug as shown by Moenkhaus (1904, *Am. J. Anat.,* 26–65). The possibility is suggested that *M. b. beryllina* may have been the form used by J. Loeb in his hybridization experiments (1912, *J. Morph.,* 23, 1–15; 1915, *Biol. Bull.,* **29**, 50–67).

Naming the hybrid embryo according to the egg used, the *M. b. beryllina* hybrid begins to lag in its developmental rate as compared with that of controls by the fifth cleavage—about three and a quarter hours at 18.5 degrees C. The *Fundulus* hybrid shows the first signs of such a lag at about 16 hours, when the expanding blastula is present in the controls. Subsequent to these stages it is rather difficult to distinguish between the effects of developmental lag and the origin of other anomalies in producing differences between hybrids and controls.

Among the anomalies observed in the hybrids are failure in yolk resorption which may be associated with other irregularities, incomplete development of the circulatory system and a rather amorphous nature to the embryo itself. Common anomalies involve the eyes. Some batches of hybrids have demonstrated such anomalies in nearly 100% of the embryos, the defects ranging from an approach of the optical organs to each other, through a perfect cyclopian condition, to a complete absence of optical structures so far as visible externally.

ERRATA

THE BIOLOGICAL BULLETIN, VOLUME 97, NO. 2, P. 260

Extrusion of jelly by eggs of Nereis limbata under electrical stimulus. W. J. V. OSTERHOUT.

In the first paragraph of the abstract noted above, "17 milliamperes" should read "170 milliamperes" and "much larger direct currents" should read "comparable direct currents."

In the third paragraph, "17 milliamperes" should read "170 milliamperes."

INDEX

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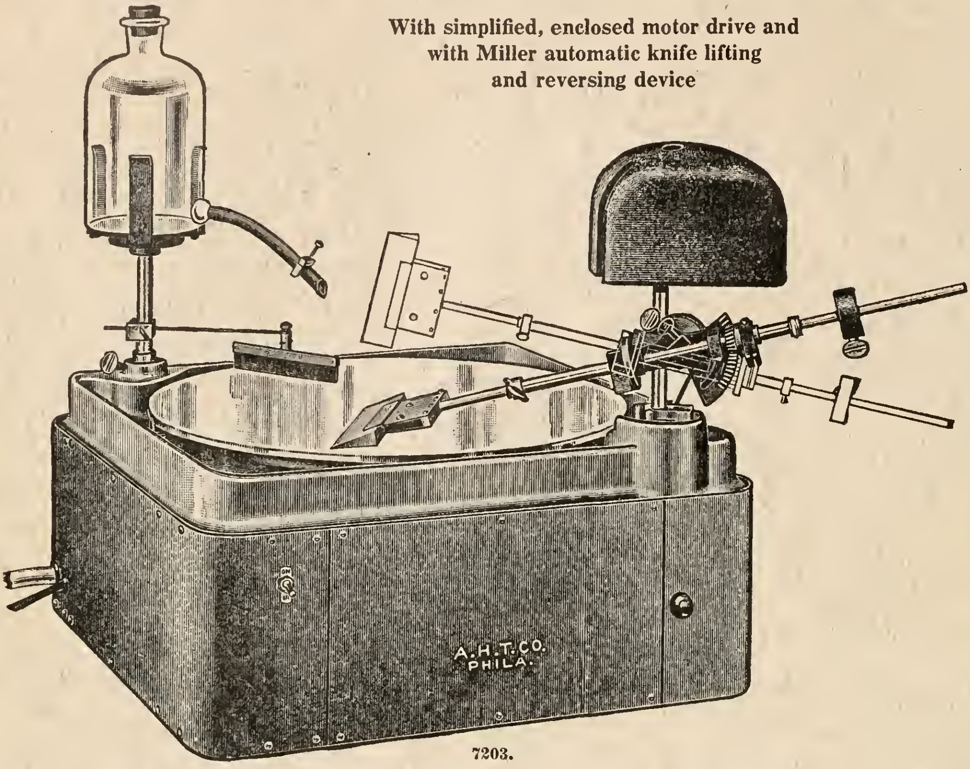

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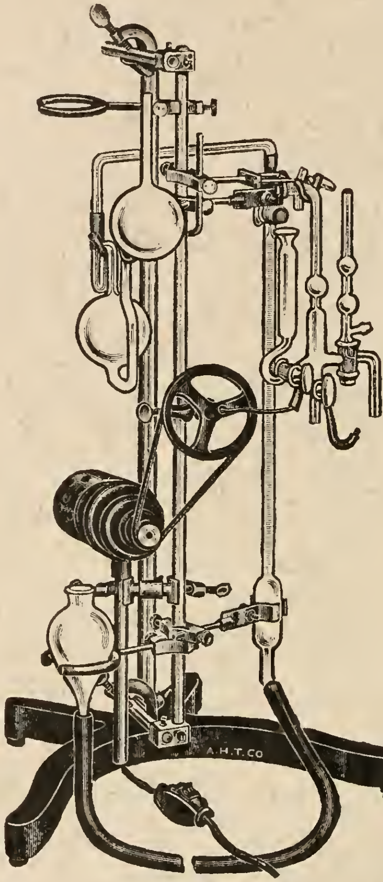

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BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

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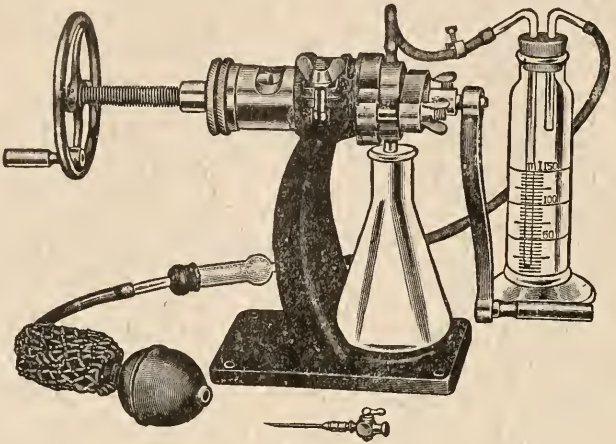

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